

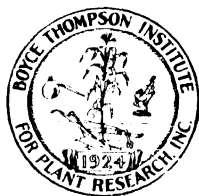
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# CONTENTS

## VOLUME II

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### No. 1, FEBRUARY, 1929

REID, MARY E. Growth of seedlings in light and in darkness in relation to available nitrogen and carbon .....	1
CARLSON, MARGERY C. Origin of adventitious roots in <i>Coleus</i> cuttings .	39
JOSEPH, HILDA C. Germination and vitality of birch seeds .....	47
SHIPPY, WILLIAM B. An inexpensive and quickly made instrument for testing relative humidity .....	72

### No. 2, MARCH, 1929

DENNY, F. E. Rôle of mother tuber in growth of potato plant .....	77
JOSEPH, HILDA C. Germination and keeping quality of parsnip seeds under various conditions .....	115

### No. 3, MAY, 1929

DENNY, F. E. Chemical changes induced in potato tubers by treatments that break the rest period .....	131
ARTHUR, JOHN M., AND NEWELL, JOHN M. The killing of plant tissue and the inactivation of tobacco mosaic virus by ultra-violet radiation	143
SHIRLEY, HARDY L. The influence of light intensity and light quality upon the growth of plants .....	159
HITCHCOCK, A. E., AND ZIMMERMAN, P. W. Effect of chemicals, temperature, and humidity on the lasting qualities of cut flowers .....	196

### No. 4, NOVEMBER, 1929

ZIMMERMAN, P. W., AND HITCHCOCK, A. E. Vegetative propagation of holly .....	205
GUTHRIE, JOHN D. Effect of environmental conditions on the chloroplast pigments .....	220
REID, MARY E. Relation of composition of seed and the effects of light to growth of seedlings .....	251
REID, MARY E. Effect of variations in the amounts of available carbon and nitrogen on the growth of wheat seedlings .....	274

## No. 5, FEBRUARY, 1930

DAVIS, W. E. Primary dormancy, after-ripening, and the development of secondary dormancy in embryos of <i>Ambrosia trifida</i> .....	285
DAVIS, W. E. The development of dormancy in seeds of cocklebur ( <i>Xanthium</i> ) .....	304
BARTON, LELA V. Hastening the germination of some coniferous seeds .....	315

## No. 6, MAY, 1930

DOBROSKY, IRENE D. Is the aster-yellows virus detectable in its insect vector? .....	343
SHIPPY, WILLIAM B. Influence of environment on the callusing of apple cuttings and grafts .....	351

## No. 7, JUNE, 1930

WILCOXON, FRANK, AND MCCALLAN, S. E. A. The fungicidal action of sulphur: 1. The alleged rôle of pentathionic acid (Herman Frasch foundation for research in agricultural chemistry. Paper no. 2) .....	389✓
DENNY, F. E., MILLER, LAWRENCE P., AND GUTHRIE, JOHN D. Enzym activities of juices from potatoes treated with chemicals that break the rest period (Herman Frasch foundation—Paper no. 3) ..	417

## No. 8, JULY, 1930

ARTHUR, JOHN M., GUTHRIE, JOHN D., AND NEWELL, JOHN M. Some effects of artificial climates on the growth and chemical composition of plants .....	445
---	-----

## No. 9, AUGUST, 1930

HARTZELL, ALBERT, AND WILCOXON, FRANK. Naphthalene fumigation at controlled concentrations (Herman Frasch foundation—Paper no. 4) .....	512
DENNY, F. E. Shortening the rest period of <i>Gladiolus</i> by treatment with chemicals (Herman Frasch foundation—Paper no. 5) .....	523
THORNTON, NORWOOD C. The use of carbon dioxid for prolonging the life of cut flowers, with special reference to roses .....	535

## No. 10, OCTOBER, 1930

PRICE, W. C. Local lesions on bean leaves inoculated with tobacco mosaic virus .....	549
--	-----

GUTHRIE, JOHN D. An iodimetric method for determining oxidase activity (Herman Frasch foundation—Paper no. 6) .....	558
HOLMES, FRANCIS O. Local and systemic increase of tobacco mosaic virus .....	563
✓ DENNY, F. E. Sucrose and starch changes in potatoes treated with chemicals that break the rest period (Herman Frasch foundation—Paper no. 10) .....	580
DENNY, F. E. The twin-leaf method of studying changes in leaves ....	592
ZIMMERMAN, P. W. Oxygen requirements for root growth of cuttings in water .....	616
Index .....	637



## GROWTH OF SEEDLINGS IN LIGHT AND IN DARKNESS IN RELATION TO AVAILABLE NITROGEN AND CARBON<sup>1</sup>

MARY E. REID

(WITH PLATES I-IV)

### Introduction

The influence of reserve substances in the plant upon its subsequent growth involves a multitude of problems of great importance, both from a practical and from a theoretical standpoint. The present investigations are concerned with the influence of certain types of reserve foods found in seeds upon the development of the seedling. A study has been made concerning the relation to growth of the amount and nature of the reserves of carbon and nitrogen which an embryo plant has at its disposal.

Some of the more practical questions which have prompted the research are as follows:

What are the responses of seedlings having available different amounts of reserve carbon and nitrogen when extra amounts of carbon and nitrogen are supplied externally? Is fertilization with nitrogen advisable in the early growth of all types of seedlings, regardless of weather and light conditions and the nitrogen reserves of the seed? Is the seedling with the larger reserves of carbon better able to live and thrive during prolonged periods of dull, cloudy weather during the early growth of the plant? Is the seedling with the larger amount of stored nitrogen at its disposal better able to withstand conditions of drought or of poor soil during the early phase of development, when nitrogen from an outside source is unavailable or available in very limited amounts?

Although the results of these experiments do not furnish answers to all these questions, it is considered that they supply considerable additional data toward this end. Previous investigations (11) with

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tomato cuttings varying in the relative proportions of the reserves of carbohydrates and nitrogen had shown very different responses when exposed to different environmental conditions, such as variations in light and darkness and the presence and absence of nitrate nitrogen in the nutrient medium. It was found that: (1) An abundant reserve of carbohydrates favored the rapid assimilation of nitrates. This was in agreement with the observations of KRAUS and KRAYBILL (8) in their study of the growth of the tomato plant. (2) An abundant reserve of carbohydrates but limited supply of nitrogen favored the growth of roots, but not that of shoots. (3) An abundance of nitrogen and an abundance of carbohydrates favored the growth of both shoots and roots. (4) An abundance of nitrogen but a limited amount of carbohydrates favored growth of shoots, especially of leaves, but not growth of roots. (5) The assimilation of nitrates occurred both in light and in darkness, but more rapidly in light, and the assimilated products were used in favoring growth of shoots especially.

The present studies have dealt with the development during the seedling stage only, and have related primarily to the influence of varying amounts of nitrogen and carbon upon the growth of shoots and roots. Experiments have been performed in light and in darkness with no nitrogen obtainable except that present in the reserves of the seeds, and also with nitrogen available in the form of nitrates. The following types of seeds were used:

Little Club wheat.....	Low protein content; high starch; low fat	{ Relatively high content of gliadin and glutenin; amide N high; rela- tively small amount of globulin protein
Vermont Champion barley....		
Giant Winter rye.....		
Illinois low-protein corn.....		
Hännchen barley (Dickinson, N.D.).....		
Blue stem wheat.....	Relatively high protein content; high starch; low fat	{ Relatively high content of gliadin and glu- tenin; high-protein corn lower in amide N and higher in globulin than low-protein type (SHOWALTER AND CARR, 14)
Marquis wheat.....		
Hännchen barley (Aberdeen, N.D.).....		
Illinois high-protein corn.....		

First of all peas .....	} Moderately high protein content; moderately high starch content; low fat (except soy bean)
Canada White peas .....	
New Era cow peas .....	
White Marrow bush beans....	
Pekin soy beans .....	
Mammoth Russian sunflower .	} Very high protein; { High globulin content; low starch;        amide N low high oil
Large Warted Hubbard squash	
Rocky Ford muskmelon .....	
Bonnie Best tomato .....	

### Experimental methods

The seeds were sterilized by immersion in a 0.25 per cent solution of uspulun. Starchy seeds were left in the solution for one hour, and those of the oily type for one-half hour, after which they were rinsed in freshly distilled water. The seeds were placed in germinators between layers of moist filter paper and allowed to sprout before being planted. This precaution was necessary since some of the seedlings were to be grown without any nitrogen from an outside source, and consequently the possibility of the seedlings obtaining nitrogenous products by the decomposition of seeds which failed to germinate had to be avoided. When the radicles had attained a length of 0.5–2 cm. the seedlings were planted in pulverized quartz and contained in 7-inch clay bulb pots. Glazed porcelain dishes with rims an inch high were used as saucers. The pots containing the sand, together with the saucers, had previously been sterilized by heating for an hour in a steam sterilizer maintained at a pressure of 15 pounds. The cultures were moistened with nutrient solutions made with salts of tested purity, and prepared according to the following formulas:

#### SOLUTION CONTAINING NITRATES

A	B
2 per cent $Mg SO_4$	4 per cent $Ca(NO_3)_2$
2 per cent $KH_2PO_4$	2 per cent $CaCl_2$
2 per cent $KNO_3$	1.5 per cent $CaSO_4$

#### SOLUTION LACKING NITRATES

A	B
2 per cent $Mg SO_4$	2 per cent $CaCl_2$
2 per cent $KH_2PO_4$	1.5 per cent $CaSO_4$
1 per cent $KCl$	

The solutions were diluted before applying them to the cultures. In preparing the solution containing nitrates, 100 cc. of solution A was made up to 1 liter with distilled water; 100 cc. of solution B was diluted in the same manner and the solutions A and B were then mixed. The solution lacking nitrates was diluted similarly. The cultures were given fresh solutions every second day in some experiments, and every third or fourth day in others. The time for making fresh applications was determined largely by light and temperature conditions, which affected the rate of growth and the ability of the seedlings to utilize the mineral nutrients. The same procedure was followed throughout the course of any one experiment. The level of the solutions in the saucers was kept nearly constant between times of applying nutrient solutions by the addition of distilled water. Freshly distilled water was used at all times. This precaution was considered necessary, since it has been shown by SEIBERT (13) that certain kinds of microscopic organisms may thrive in distilled water tanks and pipes, and that these organisms have the ability to fix atmospheric nitrogen. The external nitrogen supply was controlled in these cultures, except that ammonia in the atmosphere was not eliminated as a factor. However, all seedlings in any experiment were subjected to the same atmospheric conditions with reference to this factor, and hence it is unlikely that it can be responsible to any extent for the differences in growth that are here described. Experiments have been conducted with seedlings grown in darkness and in light with each of the two types of solutions. Except in one case (March 5-26, 1926) all experiments were repeated, and the results of the second test were found in general to be in close agreement with those of the first.

#### Growth of seedlings in darkness

The question of etiolation has enlisted the interest of a number of physiologists, whose investigations on the problem were relatively extensive in the botanical literature of fifty to sixty years ago. SACHS, G. KRAUS, GODLEWSKI, STEBLER, PFEFFER, BATALIN, RAUWENHOFF, and VINES made important contributions at this time. Still earlier, DECANDOLLE (2) had described the characteristics of the etiolated plant, and attempted, as have many others

since, to account for the peculiarities of growth in darkness. The chief characteristics of the etiolated plant as mentioned by these earlier investigators may be said to be a greatly elongated stem, and leaves much reduced in size. Cotyledons which in the light grow and develop into foliage leaves remain small and undeveloped in darkness. Of all the peculiarities of the etiolated seedlings perhaps the absence of chlorophyll is the most outstanding. Almost no mention is made of the characteristics of the roots of etiolated seedlings.

The results of some of SACHS' (12) experiments show that the great elongation of the stem and reduction in size of the leaves of etiolated plants is not without exceptions. The leaves of many monocotyledonous plants become longer in darkness than in light. Also the leaves of certain dicotyledonous plants, as *Beta*, become almost as large when grown in darkness as in light. SACHS postulated that the reason leaves failed to develop in darkness was not because of lack of food (carbohydrates supposedly), but was due to some unknown influence of chlorophyll.

DE SAUSSURE (3) was probably the first to state that leaves are dependent for their growth on the products of their own assimilation (carbohydrates inferred). G. KRAUS (9) was also of this opinion, and claimed that the leaf can only develop in darkness to the stage where it can begin to assimilate if it receives the light; if it cannot assimilate it will remain small and soon die. His only proof, however, for the fact that assimilation is essential for the growth of leaves is that he did not find starch in etiolated leaves. He recognized the fact that non-nitrogenous reserve materials as fats may be present in cotyledons, and yet the cotyledons do not grow beyond a certain relatively small size in darkness. He considered that deficiencies in cell wall formation were the chief limiting factors, therefore, and that light is necessary for the transformation of materials into cell walls. His own work (9) and that of RAUWENHOFF (10), and of a number of later investigators, have shown that light has a marked influence on the thickening of cell walls and the maturation of tissues. G. KRAUS and RAUWENHOFF thought the effect was produced by the processes of synthesis and utilization of carbohydrates. GODLEWSKI (5) claimed that the influence of light on the thickening of cell walls and its growth-retarding effect on the

growth of stems have nothing to do with the assimilation of carbohydrates. VINES (16) had previously presented data which were interpreted as proving also that the retarding influence of light on the growth of leaves is completely independent of assimilation. Both of these investigators referred to the need of uniform light for the growth of stem and leaf, rather than to the effect of strong light as compared with weaker light.

Although most of the research as to the effects of light and darkness on the growth of different organs has been conducted with seedlings, not much attention has been paid to the influence of the amount and nature of the food reserves of the seed upon the type of growth. Nevertheless such a relation was partially recognized by the investigators who studied somewhat the chemical content of the seedlings. Descriptions of the growth responses are very incomplete, however.

It has not been the province of this research to enter into a detailed study of the phenomena of etiolation, but rather to obtain some quantitative measurements of the growth of different organs, to be used for comparison with results of subsequent experiments with seedlings grown in the light.

#### 1. SEEDLINGS GROWN WITH NO EXTERNAL SOURCE OF NITROGEN

The seedlings of each kind were allowed to grow until the shoots attained their maximum length. Several preliminary tests were made to determine the most practicable method. Measurements of the heights of certain plants in each culture were made at intervals as the time of attaining maximum size approached. When no further elongation of the shoot occurred the experiment was terminated. The general appearance of the plant was taken into consideration, and slight indications of shrinking or wilting of the leaves were likewise used as an index that there was cessation of growth. The dark room in which the seedlings were grown was maintained at a nearly uniform temperature ( $21^{\circ}\text{C.}$ ), and favorable conditions of atmospheric moisture were obtained by evaporation of moisture from the cement floor covering the pebbles which was sprinkled with water daily.

In harvesting the seedlings, the roots were carefully washed

free of the quartz sand and rinsed in distilled water. Measurements of the lengths of roots and stems and of length and width of leaves and cotyledons were obtained, and also the green weights of the tissues. The quantitative results are given in table I, and illustrations of cow pea, soy bean, muskmelon, tomato, sunflower, and high- and low-protein corn seedlings are shown in figs. 1-7. Due to the fact that so many different kinds of seedlings were grown in these experiments, it was not possible to preserve the material for dry weight determinations.

Seedlings which grew from high-protein, high-oil seeds had the highest shoot to root ratios. This largely results from the development of an exceedingly long stem and a small amount of roots. The stems have a very high water content and a relatively small amount of protoplasm in the cells. Some of the seedlings of this group (for example, squash) may produce one leaf which remains very small, or no visible leaves, as in the case of tomato. Sections of the incompletely developed cotyledons showed that the tissues have a very dense compact structure. The cells remain small and intercellular spaces are only slightly developed. The similarity in behavior of these seedlings, representing three different families, suggests that the type of growth is related to the nature and quantity of reserve foods which are available.

The smallest weight of shoots in proportion to roots was found among the Gramineae. The low ratio results from the relatively greater weight of roots, the small size or lack of stems, and the extensive leaf development in which a large amount of the reserve materials has been used. The leaves of these seedlings have a much higher protoplasmic but lower water content than had the stems of the high-protein dicotyledonous seedlings previously described. Seedlings of the grass family have leaves longer and narrower, but thinner in cross-section, than those of similar seedlings grown in the light in normal atmosphere. This difference in length of leaf in light and in darkness results chiefly from the greater length of the leaf sheaths in the case of seedlings grown in darkness. The leaf blades of the high-protein types of the grass family tend to be longer in the light than in darkness, but those of the low-protein types tend to be longer in darkness than in light. Microchemical tests for starch

TABLE I  
SEEDLINGS GROWN IN DARKNESS WITHOUT NITRATES, MARCH 7-APRIL 7, 1924

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	WEIGHT OF SEEDS (GM.)	TOTAL NITROGEN IN SEEDS (%)	AVERAGE LENGTH		GREEN WEIGHTS PER PLANT			RATIO OF WEIGHTS OF SHOOTS TO ROOTS	TOTAL GROWTH PER GM. OF RESERVE FOODS (GM.)
						Shoots (cm.)	Roots (cm.)	Shoots (stems + leaves) (gm.)	Roots (gm.)	Leaf blades + cotyledons (gm.)		
Wheat.....	Blue stem	60	17	2.32	2.27	24.0	8.5	0.151	0.068	0.084	2.22	5.6
Wheat.....	Marquis	60	16	2.08	2.50	27.5	8.9	0.166	0.054	0.086	3.04	6.3
Wheat.....	Little club	60	16	1.99	1.52	20.8	15.2	0.158	0.066	0.087	2.39	6.7
Barley.....	Vermont champion	60	15	1.59	1.82	21.5	10.2	0.135	0.062	0.073	2.19	7.4
Oats.....	Storm king	60	14	1.83	2.68	21.9	11.8	0.150	0.086	0.078	1.75	7.6
Rye.....	Giant winter	60	15	1.37	1.75	19.0	9.7	0.113	0.052	0.071	2.10	7.2
Rice.....	Honduras	60	20	1.41	1.68	34.0	5.6	0.095	0.031	0.020	3.08	5.3
Corn.....	Illinois high-protein	12	14	3.27	2.76	36.0	23.7	1.470	0.686	0.696	2.14	7.9
Corn.....	Illinois low-protein	12	14	4.69	1.09	29.2	28.0	1.290	0.750	0.575	1.72	5.2
Corn.....	Illinois high-oil	12	15	3.27	.....	32.9	25.8	1.402	0.772	0.728	1.81	8.0
Corn.....	Illinois low-oil	12	15	7.31	.....	36.1	27.0	1.800	0.950	0.706	1.90	4.5
Peas.....	First of all	36	19	6.26	4.11	40.5	9.3	1.121	0.397	0.067	2.82	8.7
Peas.....	Juno	36	20	9.46	5.10	29.0	9.3	1.268	0.513	0.131	2.47	6.7
Beans.....	White Marrow bush	30	16	9.90	3.85	51.3	13.2	1.993	0.526	0.111	3.78	7.6
Cow peas.....	New era	26	13	3.39	4.40	32.1	9.6	0.757	0.237	0.058	3.19	7.6
Soy beans.....	Peking	26	17	1.75	3.40	.....	.....	0.642	0.172	0.074	3.72	12.1
Sunflower.....	Mammoth Russian	18	17	0.65	4.56	27.2	6.7	1.020	0.105	0.165	9.71	23.8
Squash.....	Hubbard Warded	14	19	2.04	5.10	28.6	13.0	2.867	0.432	0.520	6.63	23.1
Melon.....	Red Rocky Ford	20	15	0.26	5.62	14.0	7.0	0.451	0.040	0.040	6.25	22.4
Tomato.....	Bonnie Best	33	15	0.08	.....	10.1	3.2	0.040	0.005	0.007	7.20	18.8

and free-reducing substances, made on tissues of some of the seedlings just previous to harvesting, showed that many of the seedlings contained little or no starch except in the guard cells of the leaves and occasional grains in the bundle sheaths at the time the seedlings stopped growing. However, some of them contained small amounts of reducing substance.

Seeds of the Leguminosae are in certain respects intermediate to the other two groups in the chemical composition of the reserve foods, and the seedlings have a tendency to be intermediate in their responses with respect to the relative proportions of shoots to roots. Several types of seedlings of this group (for example, two kinds of peas) stopped growing before the reserves from the cotyledons were depleted. Microchemical tests showed that very little reducing substance could be found in the seedlings or in the cotyledons, but that a small amount of starch was left in the cotyledons. The supply of soluble carbohydrates in the seedling may have been insufficient to provide for further growth. It has been shown by a number of investigators that leguminous seedlings grown from high-protein seeds of especially high-protein content have insufficient carbohydrate reserves to allow a complete utilization of the stored nitrogen. The possibility of some limiting factor other than a carbohydrate should also be considered. No tests were conducted to determine whether the seedlings could be kept growing for a longer time by immersing the roots in sugar solutions.

Some variations in the relative weights of shoots and roots in relation to differences in temperature have been observed. In one series of experiments with high- and low-protein corn it was noticed that a relatively greater weight of roots was produced at 27° than at 20° C.

Observations made on cross-sections of stems and roots of the dicotyledonous seedlings showed that the processes of both nuclear and cell division have been much limited. Secondary thickening is less advanced than in stems and roots of similar seedlings grown in the light. Some microchemical tests for cell membrane substances have been made. There is some evidence that lignin is present in the xylem vessels of the stem in most of the types of seedlings. Darkness does not prevent the deposition of strengthening materials in the cell walls, although it does limit the process greatly.



## II. SEEDLINGS GROWN WITH NITRATES IN NUTRIENT SOLUTION

The quantitative data are presented in table II. When nitrates were obtainable by the growing seedlings, there was found to be a definite increase in the total green weight in thirteen of the nineteen types studied. Two of the types had the same total green weight when nitrates were available as when they were not. A decrease in the total green weight of four types was noted. Possibly in the latter forms carbohydrates were a limiting factor. There is evidence from some of the experiments to be described in a subsequent paper that the presence of nitrates in the nutrient solution stimulates respiration. This alteration in the rate of respiration of seedlings grown in darkness would have considerable effect on the duration of the reserve carbohydrates and fats.

The increases in total green weights of tissues with the use of nitrates are not nearly so great as has been found in the experiments with tomato cuttings, which had a much more abundant store of reserve carbohydrates. It was found that nitrates favored the growth of shoots much more than of roots in the experiments both with seedlings and tomato cuttings. In some of the experiments with cuttings, in which the nitrogen and carbohydrate reserves were both moderately high, the presence of nitrates in the nutrient solution in which the cuttings were grown had a definitely unfavorable effect on the growth of roots. Only four of the nineteen types of seedlings had growth of roots increased by the presence of nitrates in the nutrient solution; thirteen types had root growth somewhat suppressed; and two types had about the same weight of roots with nitrate as without. Growth of shoots was definitely favored in fourteen types of seedlings; it was inhibited in four types; and about the same in two types. These responses as to the stimulating effect of nitrates on growth of shoots also agree with results obtained with tomato cuttings.

SUMMARY OF RESULTS OBTAINED WITH SEEDLINGS GROWN  
IN DARKNESS

1. There appears to be a tendency for the relative weights of shoots to roots to vary according to the carbohydrate and nitrogen content of the seed: the higher the supply of carbohydrates in pro-

TABLE II  
SEEDLINGS GROWN IN DARKNESS WITH NITRATES AVAILABLE, MARCH 9—APRIL 7, 1925

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	WEIGHT OF SEEDS (GM.)	TOTAL NITROGEN IN SEEDS (%)	AVERAGE LENGTH		GREEN WEIGHTS PER PLANT		RATIO OF WEIGHTS OF SHOOTS TO ROOTS	TOTAL GROWTH PER GM. OF RESERVE FOODS (GM.)
						Shoots (cm.)	Roots (cm.)	Shoots (gm.)	Roots (gm.)		
Wheat.....	Blue stem	40	17	1.55	2.27	26.7	9.2	0.196	0.055	3.58	6.4
Wheat.....	Marquis	40	17	1.39	2.50	28.0	9.7	0.173	0.047	3.68	6.3
Wheat.....	Little club	40	17	1.33	1.52	24.0	9.2	0.180	0.077	2.32	7.7
Barley.....	Vermont champion	20	18	0.53	1.82	23.6	10.2	0.188	0.079	2.38	10.1
Oats.....	Storm king	31	12	0.94	2.68	25.3	9.2	0.144	0.074	1.94	7.2
Rye.....	Giant winter	52	17	1.19	1.75	22.2	10.1	0.155	0.053	2.91	9.1
Rice.....	Honduras	38	24	0.89	1.68	30.4	9.2	0.117	0.019	6.19	5.8
Corn.....	Illinois high-protein	12	22	3.27	2.76	37.1	14.8	1.340	0.427	3.13	6.5
Corn.....	Illinois low-protein	12	22	4.69	1.99	43.0	21.1	1.750	0.510	3.42	5.8
Corn.....	Illinois low-oil	12	22	7.30	.....	40.5	15.2	1.480	0.377	3.92	3.0
Peas.....	First of all	23	24	4.00	4.11	53.9	8.9	1.360	0.345	3.94	9.8
Peas.....	June	23	24	6.04	5.10	52.2	10.1	1.250	0.394	3.17	6.2
Beans.....	White Marrow bush	9	24	2.99	3.85	46.2	8.8	2.593	0.483	5.36	9.2
Cow peas.....	New era	20	23	2.61	4.40	28.5	9.3	0.725	0.177	4.08	6.9
Soy beans.....	Peking	19	29	1.28	3.40	46.2	5.1	0.822	0.098	8.35	13.6
Sunflower.....	Mammoth Russian	10	17	0.47	4.56	20.2	8.9	1.180	0.112	10.50	27.5
Squash.....	Hubbard	9	20	1.31	5.10	32.6	12.2	4.793	0.676	7.08	37.6
Melon.....	Red Rocky Ford	18	18	0.24	5.62	13.5	3.9	0.264	0.035	7.43	22.5
Tomato.....	Bonnie Best	46	18	0.12	?	12.7	1.8	0.061	0.004	16.08	25.0

portion to the nitrogen, the lower the relative weights of shoots to roots. The relation is probably not so clear as it might be if the carbohydrate reserve were not a limiting factor to the utilization of nitrogen in some of the high-nitrogen types of seedlings.

2. Nitrate nitrogen is assimilated by seedlings grown in darkness. Variations in ability to synthesize nitrates into growth-promoting substances are doubtless caused to some extent by differences in the carbohydrate or fat reserves, or in some cases by differences in both fats and carbohydrates.

3. Nitrates tend to increase growth of shoots in darkness, but they inhibit growth of roots somewhat, especially if the supply of reserve carbon compounds is much limited.

4. There is a marked inhibition in growth as to size and number of leaves in all types of seedlings investigated except among the representatives of the Gramineae. Observations indicate that this is due partly to a failure of the cells already formed to grow, although there is also a limitation of cell division. In general, the greater compactness of the foliaceous tissues, lack of intercellular spaces, and the small size of cotyledons of the foliaceous type (as in squash) show that growth of cells is inhibited.

5. Growth of roots is much restricted in practically all types of seedlings, but stem elongation is extensive with the exception of that of seedlings of the Gramineae, in which the leaf sheaths are much elongated.

6. The processes of secondary thickening of stems and roots and of deposition of materials in the cell walls are very much inhibited.

### **Seedlings grown in light in normal atmosphere**

Seasonal differences have been found to modify noticeably the growth of seedlings in the light. An attempt has been made to avoid differences caused by changes of season, such as length of day, temperature, and intensity and quality of light. The experiments on the effect of nitrogen starvation, and nitrogen feeding on the growth of different types of seedlings in the light, have been conducted chiefly during the months of October and March. During these months day length is approximately the same, except for the fact that in the October experiments the days became shorter as the experiment progressed, and in March they became longer. How-

ever, since most of the seedlings did not grow for more than three weeks, this difference in length of day at the beginning as compared with the end of the experiment could not have had much influence on the responses.

In the few tests that have been made during the months with longer days, in which the sunlight was also more intense, lower shoot to root ratios were obtained. This is to be noted to some extent by a comparison of the results shown in tables III, IV, VII, and VIII. Although the experiments were carried on during corresponding spring and fall months of the year, in the October experiment the weather was cloudy about half of the time; whereas during the March experiment there was a larger proportion of hours of sunshine. In some experiments conducted during November and the early part of December, it was noted that the shoot to root ratios were considerably higher than in the October experiment and much higher than in the March experiment.

#### EXPERIMENT I, OCTOBER 1925

Two sets of cultures were prepared in the manner described for seedlings grown in darkness. One set was given the solution lacking nitrates and the other the solution containing nitrates. The experiment was terminated when the seedlings had grown to maximum size, which was determined partially by the time of exhaustion of the food reserves, and partially by observing the time at which the shoots ceased growing. It was found that different kinds of seedlings require different periods of time to reach maximum size. The seedlings of the grass family, especially the low-protein types, attained their development in the shortest time. The cultures receiving nitrates were always harvested on the same day as the corresponding cultures not receiving extra nitrogen.

The temperature of the greenhouse was kept at 21°–22° C. during most of each 24-hour period, but on sunshiny days there was a rise during the middle of the day. All seedlings in each of the two sets of cultures were grown under the same external conditions, so such differences as are noted in the responses must be due to differences in the nature and amount of the food reserves and to the hereditary factors.

*1. Seedlings grown without extra nitrogen*

Table III gives the quantitative results of this experiment, and figs. 8-13 present illustrations of soy bean, cow pea, muskmelon, sunflower, and low- and high-protein corn seedlings. The ratios of the weights of shoots to roots were much lower than those of corresponding seedlings grown in darkness; but in agreement with the results obtained in darkness, the higher protein types of seeds produced seedlings with higher shoot to root ratios. The low-protein types, such as are found in the Gramineae, developed seedlings with the lowest shoot to root ratios. The high-protein, high-oil seeds yielded seedlings with the highest shoot to root ratios; and, as in the case of the experiments in darkness, seedlings of the Leguminosae were intermediate in their responses.

All seedlings in this experiment had an extensive development of roots in proportion to the size of the shoots. Early in the growth of the seedlings starch began to accumulate in certain tissues. The greatest ability to store starch was noted in tomato, squash, and muskmelon seedlings. It was previously shown by KRAUS and KRAYBILL (8), and later by others, that carbohydrates rapidly accumulate when nitrogen is a limiting factor for protein synthesis and growth. The non-nitrogenous carbon compounds accumulate in sunflower seedlings in the form of oils, although considerable starch is also present. In seedlings of the grass family a relatively large amount of the carbohydrate material appears to be deposited in the cell walls. All tissues of these seedlings undergo rapid differentiation, and the cell walls of the strengthening tissues rapidly increase in thickness. The tissues are physiologically old when the seedlings have grown for only two or three weeks, and the lower the nitrogen content of the seed the more rapid the maturing and eventual senescence of the tissues.

After emerging into the light, the cotyledons and leaves as they grew developed an intense green color. In some cases the color was of a darker and somewhat bluer green than that observed in the plants receiving nitrates. During the latter part of the growth period the color gradually became less intense and developed more of a yellowish tinge. These results as to amount of chlorophyll in leaves and cotyledons of seedlings receiving and not receiving nitrates

TABLE III  
SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE WITH NO EXTERNAL SOURCE OF NITROGEN, OCTOBER 7, 1925

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	TOTAL NITROGEN IN SEEDS (%)	AVERAGE LENGTH OF			GREEN WEIGHT PER PLANT			RATIO OF WEIGHTS OF SHOOTS TO ROOTS
					Shoots (cm.)	Roots (cm.)	Leaves (cm.)	Shoots (gm.)	Roots (gm.)	Leaves (gm.)	
Wheat.....	Blue stem	53	14	2.27	17.0	19.2	13.2	0.146	0.198	0.101	0.73
Wheat.....	Marquis	53	13	2.50	15.5	10.3	11.6	0.142	0.199	0.103	0.71
Wheat.....	Little club	56	13	1.52	10.7	12.8	7.7	0.096	0.149	0.053	0.64
Barley.....	Vermont champion	43	14	1.82	11.2	17.0	9.4	0.128	0.198	0.099	0.65
Oats.....	Storm king	46	18	2.68	21.0	10.9	14.5	0.171	0.268	0.115	0.64
Oats.....	Clydesdale	30	15	2.82	11.2	10.3	7.8	0.142	0.254	0.097	0.55
Rye.....	Giant winter	44	15	1.75	10.5	16.5	7.8	0.086	0.091	0.057	0.45
Rice.....	Honduras	44	22	1.68	13.2	10.5	8.6	0.056	0.069	0.023	0.81
Corn.....	Illinois high-protein	10	17	2.76	24.2	14.2	18.0	1.472	1.540	0.838	0.95
Corn.....	Illinois low-protein	10	17	1.09	14.2	11.7	9.5	0.634	0.800	0.336	0.79
Corn.....	Illinois high-oil	10	19	.....	23.5	20.0	17.0	1.312	1.775	0.740	0.73
Corn.....	Illinois low-oil	5	19	.....	20.5	15.2	15.2	1.600	1.952	0.812	0.82
Peas.....	June	18	33	5.10	17.0	12.4	2.4	1.936	1.717	.....	1.12
Peas.....	Canada white	16	23	3.60	23.7	12.1	2.4	0.947	0.890	0.456	1.06
Beans.....	White Marrow bush	5	18	3.85	15.7	11.2	6.5	3.018	1.544	1.754	1.94
Cow peas.....	New era	15	17	4.40	8.7	12.7	5.2	1.007	0.906	0.420	1.21
Soy beans.....	Peking	26	21	3.40	10.1	14.7	3.4	0.583	0.376	0.359	1.55
Sunflower.....	Mammoth Russian	22	25	4.56	15.0	14.0	3.9	1.250	0.757	0.607	1.65
Squash.....	Hubbard warted	5	33	5.10	6.3	17.0	4.5	4.124	3.032	2.610	1.36
Melon.....	Red Rocky Ford	30	33	5.62	5.2	6.1	1.3	0.371	0.303	0.221	1.22
Tomato.....	Bonnie Best	22	21	.....	2.4	7.5	0.5	0.060	0.044	0.038	1.36

agree with those reported by DEUBER (4) for soy bean seedlings. Some of the seedlings had traces of red color in the leaves. Definite changes in pigmentation developed during the growth of the corn seedlings. The leaves of the high-protein corn seedlings had no red color except traces in the midrib at the time of harvest. The low-protein corn seedlings, on the contrary, had much red in the leaves, were much less green, and especially were less bluish-green. The stems of both the high- and low-protein seedlings were very red. Although no red color developed in the wheat seedlings, there were similar differences in the amount and shade of green in the leaves of the high-protein (Marquis) and low-protein (Little Club) types.

## *2. Seedlings grown with nitrates*

The data are presented in table IV. Most of the seedlings began to synthesize nitrates at an early stage of growth. This was especially true of seedlings of the grass family. The leguminous types did not use nitrates rapidly. The composition of the nutrient solution may not have been so well adapted for the growth of seedlings of this family as for the other kinds grown. Also these seedlings may be more sensitive to light conditions than some of the other types, and may require a greater amount of light to enable them to effect a rapid synthesis of nitrates into growth-promoting substances. In some other experiments performed during April and May, when light was of greater intensity and the days were longer, there was a much more rapid synthesis of nitrates by leguminous seedlings as indicated by growth. It may also be possible that, in the sterile cultures, the lack of nodules containing the nitrogen-fixing bacteria may have contributed somewhat in making these seedlings less efficient than the other types in the metabolism of nitrates.

Table V summarizes the effects of the utilization of nitrates on the growth of various organs. There is a striking difference in the effect of nitrates on the growth of shoots and roots. Nitrates increased growth of shoots remarkably, whereas eleven of the nineteen types grown had the total green weight of roots very little affected, and of these eleven types, five varied less than 5 per cent from the weights of roots of seedlings not receiving nitrates, and in four types the roots weighed less when nitrates were present than when they

TABLE IV  
SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE WITH NITRATES, OCTOBER 7, 1925

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	TOTAL NITROGEN (%)	AVERAGE LENGTH OF			GREEN WEIGHT PER PLANT			RATIO OF WEIGHTS OF SHOOTS TO ROOTS
					Shoots (cm.)	Roots (cm.)	Leaves (cm.)	Shoots (gm.)	Roots (gm.)	Leaves (gm.)	
Wheat.....	Blue stem	53	14	2.27	23.0	15.2	19.0	0.277	0.213	0.197	1.30
Wheat.....	Marquis	53	13	2.50	22.5	15.2	18.0	0.262	0.198	0.180	1.32
Wheat.....	Little club	56	13	1.52	19.7	14.5	13.4	0.205	0.197	0.148	1.04
Barley.....	Vermont champion	43	14	1.82	22.6	14.2	17.4	0.355	0.186	0.257	1.93
Oats.....	Storm king	46	18	2.68	28.0	9.2	21.5	0.405	0.274	0.262	1.47
Oats.....	Clydesdale	30	15	2.82	19.5	11.7	14.0	0.220	0.270	0.190	1.22
Rye.....	Giant winter	44	15	1.75	18.7	13.6	14.2	0.242	0.175	0.173	1.38
Rice.....	Honduras	44	22	1.68	8.1	6.3	4.7	0.060	0.060	0.026	1.01
Corn.....	Illinois high-protein	10	17	2.76	32.2	16.7	24.3	2.587	1.468	1.472	1.36
Corn.....	Illinois low-protein	10	17	1.09	26.8	17.0	19.4	1.946	1.547	1.049	1.25
Corn.....	Illinois high-oil	10	19	.....	31.6	16.5	24.0	2.512	2.559	1.507	0.98
Corn.....	Illinois low-oil	5	19	.....	30.4	14.2	23.2	2.776	2.582	1.618	1.07
Peas.....	Juno	18	33	5.10	20.0	14.2	3.0	2.730	0.815	.....	1.50
Peas.....	Canada white	16	23	3.60	21.6	13.2	2.5	1.348	0.897	.....	1.50
Beans.....	White Marrow bust.	5	18	3.85	15.2	12.0	8.0	4.260	2.120	2.610	2.01
Cow peas.....	New era	15	17	4.40	11.0	13.0	6.0	1.658	1.068	0.978	1.62
Soy beans.....	Peking	26	21	3.40	11.3	11.4	3.6	0.791	0.449	0.513	1.76
Sunflower.....	Mammoth Russian	22	25	4.56	24.2	10.1	7.6	4.788	1.328	2.632	3.60
Squash.....	Hubbard warted	5	33	5.10	13.9	10.6	10.4	12.840	3.920	6.804	3.27
Melon.....	Red Rocky Ford	30	33	5.62	7.5	7.3	3.5	0.782	0.307	0.456	2.55
Tomato.....	Bonnie Best	22	21	.....	2.5	7.5	5.2	0.296	0.074	0.206	4.00



TABLE V  
EFFECT OF UTILIZATION OF NITRATES UPON GROWTH OF SEEDLINGS KEPT IN NORMAL ATMOSPHERE

PLANT	VARIETY	NO. OF PLANTS	LENGTH OF GROWTH PERIOD (DAYS)	TOTAL NITROGEN IN SEEDS (%)	TOTAL WEIGHT OF SEEDS (GM.)	PERCENTAGE INCREASE IN GREEN WEIGHT DUE TO UTILIZATION OF NITRATES			
						Total	Shoots	Roots	Leaves
Wheat.....	Blue stem	53	14	2.27	2.05	+ 42.0	+ 89.0	+ 7.3	+ 94.9
Wheat.....	Marquis	53	13	2.50	1.84	+ 34.4	+ 83.8	- 0.8	+ 83.3
Wheat.....	Little club	50	13	1.52	1.86	+ 03.6	+ 112.9	+ 30.7	+ 170.3
Barley.....	Vermont champion	43	14	1.82	1.14	+ 66.8	+ 174.6	- 4.3	+ 158.1
Oats.....	Storm king	46	18	2.68	1.40	+ 54.5	+ 136.7	+ 2.2	+ 136.9
Oats.....	Clydesdale	30	15	2.82	1.35	+ 23.7	+ 90.1	+ 13.3	+ 95.9
Rye.....	Giant winter	44	15	1.75	1.01	+ 50.4	+ 182.0	- 8.5	+ 201.9
Rice.....	Honduras	44	22	1.68	1.04	- 3.8	+ 7.7	- 13.2	+ 14.8
Corn.....	Illinois high-protein	10	17	2.76	2.73	+ 48.7	+ 75.7	+ 22.8	+ 75.1
Corn.....	Illinois low-protein	10	17	1.09	3.91	+ 143.5	+ 206.9	+ 93.4	+ 212.2
Corn.....	Illinois high-oil	10	19	.....	2.73	+ 64.2	+ 60.9	+ 44.1	+ 103.6
Corn.....	Illinois low-oil	5	19	.....	3.04	+ 50.8	+ 73.5	+ 32.2	+ 99.2
Peas.....	Juno	18	33	5.10	4.73	+ 24.4	+ 40.9	+ 5.7	.....
Peas.....	Canada white	16	23	3.60	2.33	+ 22.3	+ 42.3	+ 0.8	.....
Beans.....	White Marrow bush	5	18	3.85	1.66	+ 39.8	+ 41.1	+ 37.3	+ 46.5
Cow peas.....	New era	15	17	4.40	1.95	+ 29.1	+ 49.3	+ 11.2	+ 133.0
Soy beans.....	Peking	26	21	3.40	1.75	+ 29.6	+ 35.6	+ 19.4	+ 42.7
Sunflower.....	Mammoth Russian	22	25	4.56	1.04	+ 204.6	+ 282.9	+ 75.4	+ 333.1
Squash.....	Large Hubbard	5	33	5.10	0.73	+ 133.7	+ 211.4	+ 29.2	+ 106.6
Muskmelon.....	warted	30	33	5.62	0.40	+ 61.5	+ 111.0	+ 1.3	+ 106.3
Tomato.....	Red Rocky Ford	22	21	.....	0.06	+ 255.7	+ 393.3	+ 68.2	+ 442.1
	Bonnie Best			.....					

were not. It is true, however, that the roots of most of the seedlings receiving nitrates had somewhat more branching than those of the plants starved for nitrogen. The roots of most of the nitrated plants were shorter. The strengthening tissues of both roots and stems in most of the seedlings had somewhat less thickening of the cell walls, and the storage carbohydrates were in all cases less abundant than in the plants starved for nitrogen. The total increase due to the use of nitrates was greater for the high-protein, high-oil types of seedlings, but this was partially because these seedlings grew for a longer time. Of the three kinds of wheat grown, the starchy low-protein type (Little Club) made the greatest gain with the use of nitrates; and in the case of corn the starchy low-protein made a greater gain than the less starchy high-protein type.

The influence of light on the growth of different parts of seedlings is shown in table VI.

*Effect of light on plants grown without extra nitrogen*

The total green weight increased in plants grown without nitrogen, especially in the high-protein forms. The low-protein starchy types gained only slightly. The green weights of stem plus petioles all decreased under the influence of light, and the decreases ranged from 3.1 per cent in the case of stems of cow pea seedlings to 65.1 per cent in the case of stems of soy bean seedlings. There appeared to be no difference between the high- and low-protein types in the responses of stems and petioles to light. In all these experiments the leaf sheaths and petioles have been included with stem tissue, as they were considered to approach more nearly to stem than to leaf tissues in content and composition. The leaves and cotyledons gained in green weight in the light in most cases; however, the leaves of the starchy low-protein seedlings decreased. This loss in weight amounted to 37.8 per cent for Little Club wheat and 41.6 per cent for Illinois low-protein corn. In a general way it may be stated that the higher the nitrogen content of the seed, the greater the increase in green weight of leaves due to the influence of light. The leaves of leguminous seedlings were very responsive, and the growth of leaves of the high-protein, high-oil seedlings was remarkably influenced by light.

TABLE VI  
PERCENTAGE INCREASE OR DECREASE IN WEIGHT OF VARIOUS ORGANS DUE TO INFLUENCE OF LIGHT, OCTOBER 1925

PLANT	VARIETY	PLANTS GROWN WITHOUT NITRATES					PLANTS GROWN WITH NITRATES AVAILABLE			
		Total	Shoots	Stems + petioles	Leaves + cotyledons	Roots	Total	Shoots	Roots	
Wheat	Blue stem	+ 57.1	- 3.3%	- 32.8%	+ 20.2%	+ 101.2	+ 95.2	+ 41.3%	+ 287.3	
Wheat	Marquis	+ 55.0	- 14.4	- 51.2	+ 19.8	+ 268.5	+ 109.0	+ 51.4	+ 321.3	
Wheat	Little club	+ 9.3	- 39.2	- 39.4	- 37.8	+ 125.8	+ 56.4	+ 13.8	+ 155.8	
Barley	Vermont champion	+ 65.5	- 5.1	- 53.2	+ 35.6	+ 210.3	+ 103.8	+ 88.8	+ 225.3	
Oats	Storm king	+ 86.0	+ 14.0	- 22.2	+ 47.4	+ 211.6	+ 211.4	+ 181.2	+ 270.2	
Rye	Giant winter	+ 7.2	- 23.9	- 30.9	- 19.7	+ 75.0	+ 100.5	+ 56.1	+ 230.2	
Rice	Honduras	- 0.8	- 41.0	- 56.0	+ 15.0	+ 122.6	- 49.1	- 48.7	+ 215.8	
Corn	Illinois high-protein	+ 39.7	+ 0.1	- 18.9	+ 20.4	+ 124.5	+ 129.4	+ 93.0	+ 243.8	
Corn	Illinois low-protein	- 29.7	- 50.8	- 58.3	- 41.6	+ 6.6	+ 54.6	+ 11.2	+ 203.3	
Corn	Illinois high-oil	+ 42.0	- 6.4	- 15.1	+ 1.6	+ 120.9	.....	.....	.....	
Corn	Illinois low-oil	+ 29.1	- 11.1	- 27.9	+ 15.0	+ 105.4	+ 187.9	+ 87.5	+ 584.8	
Peas	Juno	+ 105.1	+ 52.6	.....	.....	+ 234.7	+ 176.4	+ 118.4	+ 360.6	
Beans	White Marrow bush	+ 81.1	+ 51.4	- 32.8	+ 1480.1	+ 193.5	+ 107.4	+ 64.2	+ 338.9	
Cow peas	New era	+ 101.5	+ 44.9	- 3.1	+ 624.1	+ 282.3	+ 195.5	+ 128.6	+ 469.5	
Soy beans	Peking	+ 17.8	+ 9.2	- 65.1	+ 432.6	+ 118.6	+ 34.8	- 3.7	+ 358.2	
Sunflower	Mammoth Russian	+ 78.4	+ 22.5	- 36.9	+ 208.0	+ 620.9	+ 373.3	+ 395.7	+ 1085.7	
Squash	Hubbard warted	+ 116.0	+ 43.8	- 47.2	+ 401.9	+ 601.8	+ 266.4	+ 167.9	+ 479.6	
Melon	Rocky Ford	+ 131.6	+ 47.8	- 40.2	+ 449.7	+ 656.0	+ 264.2	+ 196.2	+ 777.1	
Tomato	Bonnie Best	+ 131.1	+ 50.0	- 45.0	+ 387.2	+ 780.0	+ 499.2	+ 385.2	+ 1750.0	

One of the most noticeable effects of light is that of the strongly positive influence which it exerts on the growth of roots. The low-protein starchy types had this stimulating effect of light on growth of roots much less than all other kinds of seedlings. The roots of starchy low-protein corn seedlings gained 6.6 per cent by the action of light, the roots of rye 75 per cent, and Little Club wheat 125 per cent. Light caused roots of leguminous seedlings to gain from 119 to 282 per cent. The roots of the higher protein seedlings had much greater increases. The gains in weights of roots of high-protein, high-oil seedlings ranged from 602 to 780 per cent.

*Effect of light on plants receiving nitrates*

The total green weight was increased in the nitrated plants exposed to light as compared with those grown in darkness, with the exception of rice, in which there was no increase either in weight or in size. This feature of the behavior of rice has been pointed out previously. A peculiarity in the growth of the rice seedlings in darkness is that plants receiving and not receiving nitrates both had very much longer shoots than the corresponding plants grown in the light. Light caused an increase in growth of shoots (stems plus leaves) in most types of seedlings, and growth of roots was greatly increased in all of them. The gains in weights of roots ranged from 155 per cent for Little Club wheat to 1750 per cent for tomato seedlings.

EXPERIMENT II, MARCH 5-26, 1926

This experiment was conducted to determine the weekly increments of growth made by various types of seedlings when receiving and not receiving nitrate nitrogen. In the previous experiment it had been found that different seedlings required different lengths of time to use the reserve nitrogen of the seed, and since the seedlings receiving nitrates were harvested at the same time as seedlings starved for nitrogen, it was difficult to compare one type with another because of the different lengths of the period of growth. This experiment was planned to overcome this difficulty. This kind of an experiment, although better for studying the ability of various types of seedlings to utilize nitrates at different stages of germination and early growth, is not so good for comparing the ability of different types to grow

without extra nitrogen. When seedlings of the grass family were two weeks of age at this season of the year many of them had almost ceased growing, although they were still increasing slightly in weight. The higher protein types were still growing by the end of the third week and had not then attained maximum size.

### *1. Seedlings not receiving nitrates*

The quantitative results are given in table VII. Illustrations of tomato and sunflower seedlings are shown in figs. 14, 16, 18, 20, 22, 24, 26, and 28. The weight of shoots in proportion to that of roots tended to decrease week by week. The relative proportion of shoots to roots of seedlings grown from starchy seeds diminished conspicuously during the second week, but there was relatively little change during the third week. The same situation was found with the higher protein seedlings, with the exception of squash, which grew relatively slowly during the first week. As in the previous experiment, the high-protein seedlings had higher shoot to root ratios than the low-protein seedlings. With the exception of the high-protein barley (Dickinson, N.D.), the greatest gains in green weight were made by the roots of all types of seedlings during the second week. Shoots of seedlings of the grass family and of sunflower grew most rapidly during the first week, and those of the leguminous type and squash and tomato during the second week.

### *2. Seedlings receiving nitrates*

The quantitative results are shown in table VIII, and illustrations of tomato and sunflower seedlings in figs. 15, 17, 19, 21, 23, 25, 27, and 29. The shoot to root ratios were higher in the cultures receiving nitrates than in those not receiving them during the entire period of growth, although in several instances the differences at the end of the first week were not appreciable. The ratios of shoots to roots of the nitrated plants tended to become progressively higher week by week, whereas those of the nitrogen-limited plants became progressively lower. With the exception of seedlings of the legumes, there were marked increases in length of shoots and decreases in length of roots with the use of nitrates. Most of the nitrated plants had more branching of the roots, and the leaves were longer and wider.

PLANT	VARIETY	No. of PLANTS	TOTAL NITRO-GEN IN SEEDS (%)	ONE WEEK			TWO WEEKS			THREE WEEKS			AVERAGE LENGTH AT END OF THIRD WEEK	GREEN WEIGHT OF TISSUE PRODUCED AT END OF THIRD WEEK PER 0.1 GM. OF RESERVE NITROGEN					
				Weight of shoots (gm.)	Ratio of weights of shoots to roots	Weight of shoots (gm.)	Ratio of weights of shoots to roots	Weight of shoots (gm.)	Ratio of weights of shoots to roots	Weight of shoots (gm.)	Ratio of weights of shoots to roots								
Peas	Marquis	62	2.50	0.089	0.105	0.85	0.134	0.233	0.57	0.206	0.332	0.58	15.8	14.0	12.6	56.7	20.95	35.74	
	Little club	62	1.52	0.062	0.103	0.66	0.085	0.219	0.39	0.133	0.322	0.41	11.2	13.9	8.8	99.6	20.15	79.42	
	Clyde-dale	62	2.82	0.096	0.110	0.88	0.144	0.244	0.58	0.107	0.207	0.56	16.4	15.8	13.2	30.6	13.20	23.46	
	Hanchen (Dickinson)	62	2.76	0.155	0.247	0.62	0.205	0.340	0.60	0.207	0.381	0.70	21.5	15.2	15.2	69.0	28.43	40.58	
	Hanchen (Aberdeen)	62	2.20	0.138	0.156	0.88	0.185	0.345	0.53	0.201	0.376	0.53	17.6	17.6	12.6	65.0	22.65	42.38	
	New era	10	4.40	0.295	0.117	2.52	0.772	0.660	1.18	(-5)	0.985	0.62	6.2	11.3	4.0	27.9	(-5)	17.22	
Peas	First of all	32	4.11	0.110	0.141	0.78	0.570	0.540	1.06	0.610	1.120	1.05	20.1	13.2	2.5	32.1	16.49	15.66	
	Peking	32	3.40	0.359	0.127	2.81	0.590	0.313	1.88	1.180	0.630	0.459	11.2	12.0	3.7	47.2	27.57	19.69	
	Hubbard	14	5.10	2.04	0.693	0.338	2.863	1.163	2.48	3.750	2.581	1.45	7.5	18.2	4.3	85.2	59.48	34.75	
	Mammoth Russian	20	4.56	0.95	0.635	0.257	2.47	0.943	0.575	1.64	1.210	0.976	1.24	11.9	11.3	3.4	100.0	55.89	45.08
	Tomato	62	.....	0.16	0.030	0.013	0.070	0.046	1.51	0.075	0.073	1.02	2.7	10.1	1.2	123.6	93.5	62.1	

TABLE VIII

SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE AND HARVESTED AT END OF ONE, TWO, AND THREE WEEKS OF GROWTH,  
WITH NITRATES, MARCH-APRIL, 1926

PLANT	VARIETY	No. OF GEN. IN PLANTS	TOTAL NITRO-GEN IN SEEDS (%)	WEIGHT OF SEEDS (gm.)	ONE WEEK			TWO WEEKS			THREE WEEKS			AVERAGE LENGTH AT END OF THIRD WEEK		GREEN WEIGHT OF TISSUE PRODUCED PER 0.1 CM. OF RESERVE NITROGE			
					Weight of shoots (gm.)	Weight of roots (gm.)	Ratio of weights of shoots to roots	Weight of shoots (gm.)	Weight of roots (gm.)	Ratio of weights of shoots to roots	Weight of shoots (gm.)	Weight of roots (gm.)	Ratio of weights of shoots to roots	Shoots (cm.)	Roots (cm.)		Leaves (cm.)	Total Shoots (gm.)	Total Roots (gm.)
Wheat	Marquis	62	2.50	2.44	0.150	0.113	1.32	0.408	0.279	1.46	0.891	0.338	2.72	36.6	8.3	27.8	124.1	90.81	33.
Wheat	Little club	62	1.52	1.87	0.097	0.115	0.84	0.277	0.239	1.15	0.668	0.358	2.01	31.6	11.2	25.2	218.1	143.8	72.
Oats	Clydesdale	62	2.82	2.78	0.149	0.150	0.99	0.394	0.269	1.47	0.871	0.378	2.31	42.9	13.9	29.1	91.1	68.9	25.
Barley	Flintshire	62	2.76	2.11	0.210	0.212	0.99	0.665	0.362	1.83	1.120	0.447	2.73	37.0	10.7	30.4	166.0	110.3	47.
Barley	Hammerhead	62	2.20	2.50	0.233	0.201	0.86	0.731	0.379	1.92	1.110	0.443	2.57	37.8	8.2	30.3	173.0	125.1	48.
Cow peas	New era	10	4.40	1.30	0.332	0.130	2.55	1.339	0.804	1.66	1.300	0.490	1.77	5.0	8.2	5.0	41.9	21.3	17.
Peas	First of all	32	4.11	5.57	0.123	0.134	0.92	0.915	0.656	1.39	1.351	0.681	1.98	3.5	29.1	5.0	48.4	18.0	9.
Soy bean	Peking	32	3.40	2.15	0.360	0.166	3.41	0.702	0.300	2.33	0.886	0.583	1.70	13.0	10.0	4.0	61.2	38.5	22.
Squash	Hubbard	14	5.10	2.04	1.579	0.468	3.37	6.107	1.736	3.52	14.500	3.463	4.26	35.4	17.0	70.0	211.0	105.2	45.
Sunflower	Mammoth Russian	20	4.50	0.95	0.714	0.245	2.91	2.011	0.611	3.29	4.666	0.901	5.16	32.8	11.3	8.2	72.2	53.0	10.
Tomato	Bonnie Best	62	.....	0.16	0.067	0.015	4.40	0.434	0.061	7.15	1.135	0.134	8.46	20.2	8.1	7.5	250.8	115.2	41.

Both shoots and roots of all the types of seedlings made the greatest gains in green weight during the second week. At the end of the first week the nitrated seedlings of the grass family had greener leaves than the seedlings not receiving nitrogen. Oat seedlings were an exception in this respect. There was no difference in the greenness of the two sets of leaves in the three types of leguminous seedlings. This condition was correlated with little difference in size of the nitrated and un-nitrated cultures. There was very little difference in color of the cotyledons of tomato, squash, and sunflower seedlings of the two sets of cultures at the end of the first week.

At the end of the second week the first leaves of seedlings of the grass family which were not receiving nitrates were beginning to die at the tips, and the general color of the leaves was a yellower green than that of the nitrated seedlings. With the exception of cow pea seedlings, in which there was no difference in color, the leguminous seedlings not receiving nitrates had greener leaves at the end of the second week. The cotyledons and leaves of sunflower and squash and the cotyledons of tomato seedlings not receiving nitrates were greener than those of plants receiving nitrates.

At the end of the third week all plants receiving nitrates had greener leaves than those of the nitrogen-starved plants.

It has been found in both the March (1925) and October (1926) experiments that seedlings of the grass family developed signs of starvation for nitrogen earlier than the higher protein types. Seedlings from the latter type of seeds had the capacity to continue growth for a longer time. There also appeared to be a difference in the amount of green tissue produced per 0.1 gm. of reserve nitrogen. The results of the October experiment are given in table IX, and of the March experiment in tables X and XI. The four types of high-protein, high-oil seedlings differ from all the others in this respect. Per unit of reserve nitrogen their total green weight is greater, and the difference in weight as compared with seedlings grown from the low-protein seeds is brought about by a greater growth of stems and leaves. There is no distinct difference in the weight of roots produced per unit of nitrogen by the different classes of seedlings. Just what is the cause or significance of this increased growth by the high-protein, high-oil seedlings cannot be stated. The high-protein, high-oil types



TABLE IX

GREEN WEIGHT OF TISSUE IN GRAMS PRODUCED PER 0.1 GM. OF RESERVE NITROGEN BY SEEDLINGS GROWN WITH NO EXTERNAL SOURCE OF NITROGEN, MARCH-APRIL, 1924

PLANT	VARIETY	AMOUNT OF N PER SEED (MG.)	DARKNESS				LIGHT					
			Total	Shoots (stems + leaves)	Stems + petioles	Leaves + cotyle- dons	Roots	Total	Shoots (stems + leaves)	Stems + petioles	Leaves + cotyle- dons	Roots
Wheat.....	Blue stem	0.878	24.9	17.2	7.8	9.4	7.7	35.4	15.0	4.62	10.4	20.4
Wheat.....	Marquis	0.982	22.4	16.9	8.2	8.7	5.5	32.4	13.5	3.73	9.76	18.9
Wheat.....	Little club	0.504	44.4	31.3	14.1	17.2	13.1	48.6	19.0	8.53	10.5	29.6
Barley.....	Vermont champion	0.482	40.9	28.0	12.9	15.1	12.8	62.0	24.4	5.48	18.9	37.6
Oats.....	Storm king	0.817	28.8	18.3	8.8	9.5	10.5	49.1	19.1	6.21	12.9	30.0
Rye.....	Giant winter	0.401	41.1	28.1	10.4	17.7	12.9	61.9	19.2	6.39	12.8	42.7
Rice.....	Honduras	0.396	31.7	24.0	19.0	5.0	7.8	29.0	13.0	7.67	5.34	16.0
Corn.....	Illinois high-protein	7.53	28.6	19.5	10.3	9.2	9.1	40.0	19.5	8.42	11.1	20.4
Corn.....	Illinois low-protein	4.26	47.8	30.2	16.7	13.5	17.6	33.7	14.9	6.99	7.88	18.8
Peas.....	Juno	13.4	13.3	9.4	8.5	0.9	3.8	25.6	13.6	6.29	7.30	12.0
Beans.....	White Marrow bush	12.8	19.7	15.5	14.6	0.9	4.1	32.3	21.4	8.95	12.4	10.9
Cow peas.....	New era	5.74	17.2	13.0	12.9	0.1	4.1	26.8	14.7	9.05	5.61	12.1
Soy beans.....	Peking	2.29	35.5	28.0	25.1	2.9	7.5	39.6	24.1	9.25	14.7	15.5
Sunflower.....	Mammoth Russian	2.17	51.8	47.0	39.4	7.6	4.8	80.3	50.0	25.7	24.3	30.3
Squash.....	Hubbard	9.83	33.5	29.1	23.8	5.3	4.4	67.9	39.1	14.7	24.8	28.8
Melon.....	Red Rocky Ford	0.753	38.6	33.3	28.0	5.3	5.3	83.4	45.9	18.6	27.3	37.5
Tomato.....	Bonnie Best	0.117	38.4	34.2	27.6	6.6	4.2	80.0	51.2	18.7	32.5	37.6

TABLE X

GREEN WEIGHT OF TISSUES IN GRAMS PRODUCED PER 0.1 CM. OF RESERVE NITROGEN BY SEEDLINGS HAVING NO EXTERNAL SOURCE OF NITROGEN, MARCH 5-26, 1926

PLANT	VARIETY	NO. OF PLANTS	TOTAL NITROGEN IN SEEDS (%)	WEIGHT OF SEEDS (gm.)	ONE WEEK			TWO WEEKS			THREE WEEKS		
					Total	Shoots	Roots	Total	Shoots	Roots	Total	Shoots	Roots
Wheat.....	Marquis	62	2.50	2.44	20.50	9.83	10.72	37.40	13.67	23.70	56.70	20.95	35.74
Wheat.....	Little club	62	1.52	1.87	32.40	12.17	20.22	59.82	16.80	43.07	89.70	26.24	63.38
Oats.....	Clydesdale	62	2.82	2.78	16.30	7.50	8.67	30.7	11.40	19.28	36.6	13.20	23.46
Barley.....	Hännchen (Dickinson)	62	2.76	2.11	42.80	16.48	26.32	58.10	21.84	36.22	69.00	28.43	40.58
Barley.....	Hännchen (Aberdeen)	62	2.20	2.50	33.10	15.56	17.58	59.70	20.85	38.89	65.00	22.65	42.38
Cow peas.....	New era	10	4.40	1.30	7.20	5.16	2.04	25.00	13.49	11.54	27.90	5 leaves had dropped off to 66	17.22
Peas.....	First of all	32	4.11	5.57	3.50	1.54	1.96	15.5	7.97	7.55	32.1	16.49	15.66
Soy bean.....	Peking	32	3.40	2.15	21.3	15.7	5.58	39.5	25.83	13.71	47.2	27.57	10.69
Squash.....	Hubbard	14	5.10	2.04	13.0	9.33	4.55	54.6	38.94	15.65	85.2	50.48	34.75
Sunflower.....	Mammoth Russian	20	4.56	0.95	41.2	29.33	11.87	70.1	43.57	26.56	100.9	55.89	45.08
Tomato.....	Bonnie Best	62	0.00729 gm. in 62 seeds	.....	36.8	25.6	11.2	99.0	59.5	39.5	125.6	63.5	62.1

TABLE XI

GREEN WEIGHT OF TISSUES IN GRAMS PRODUCED PER 0.1 GM. OF RESERVE NITROGEN BY SEEDLINGS RECEIVING NITRATES,  
MARCH 3-26, 1926

PLANT	VARIETY	NO. OF PLANTS	TOTAL NITROGEN IN SEEDS (%)	WEIGHT OF SEEDS (GM.)	ONE WEEK			TWO WEEKS			THREE WEEKS		
					Total	Shoots	Roots	Total	Shoots	Roots	Total	Shoots	Roots
Wheat.....	Marquis	62	2.50	2.44	26.82	15.28	11.54	69.92	41.54	28.38	124.1	90.81	33.34
Wheat.....	Little club	62	1.52	1.87	46.26	21.12	25.14	112.5	60.38	52.18	218.1	145.8	72.35
Oats.....	Clydesdale	62	2.82	2.78	23.71	11.83	11.88	52.43	31.17	21.26	94.10	68.87	25.19
Barley.....	Hannchen (Dickinson)	62	2.76	2.11	44.91	22.37	22.54	109.5	70.84	38.66	166.9	119.3	47.61
Barley.....	Hannchen (Aberdeen)	62	2.20	2.50	55.67	26.25	20.42	125.1	82.40	42.70	173.9	125.1	48.80
Cow peas.....	New era	10	4.40	1.30	8.07	5.80	2.27	37.46	23.41	14.05	41.90	24.30	17.64
Peas.....	First of all	32	4.11	5.57	3.59	1.72	1.87	21.95	12.79	9.16	28.40	18.91	9.51
Soy bean.....	Peking	32	3.40	2.15	20.37	15.75	4.62	43.89	30.72	13.17	61.20	38.52	22.66
Squash.....	Hubbard	14	5.10	2.04	27.55	21.25	6.30	105.6	82.21	23.36	241.0	195.2	45.82
Sunflower.....	Mammoth Russian	20	4.56	0.95	44.28	32.97	11.31	121.1	92.88	28.22	256.8	215.2	41.61
Tomato.....	Bonnie Best	62	0.00729 gm. in 62 seeds	.....	70.0	57.2	12.8	423.0	370.9	52.1	1084.5	970.0	114.5

of seedlings also have much the greatest efficiency in the metabolism of inorganic nitrogen.

### Discussion

The questions raised in the introduction will now be considered.

1. What are the responses of seedlings having different amounts of reserve carbon and nitrogen when extra amounts of carbon and nitrogen are supplied externally?

The relations of varying amounts of available carbon and nitrogen to the growth of shoots and roots of seedlings are much like those found with tomato cuttings. Seedlings grown from seeds having large carbon reserves in proportion to the nitrogen tend to have low shoot to root ratios when grown without an external source of nitrogen, and those grown from seeds having relatively large amounts of nitrogen have higher shoot to root ratios if the seedlings are grown in the light. If the seedlings are grown in darkness there appears to be a somewhat similar, although considerably less definite, relation between the type of growth and the kind and amount of the food reserves.

The quantitative differences in the growth of different organs of seedlings kept in the light appear to be more directly related to the amounts of available carbon and nitrogen than to genetical affiliations. Within the grass family, for example, we find variations in growth related to the amount of stored foods. The very starchy, low-protein types of wheat, barley, and corn have lower shoot to root ratios than the corresponding somewhat less starchy and higher protein types. The results obtained with the four kinds of seedlings grown from high-protein, high-oil seeds, representing three different families, also indicate that the responses are related to the types and quantities of reserve foods.

When extra nitrogen is supplied, growth is in most cases slightly increased in darkness and is greatly increased in the light. The extra nitrogen favors growth of shoots more than that of roots. The roots of the nitrated seedlings are shorter. In no case was the increase with the use of nitrates in darkness equal to that obtained with the very high-carbohydrate tomato cuttings. It is supposed that this quantitative difference in the responses of seedlings as

compared with the cuttings resulted from the smaller amount of carbohydrate in proportion to the stored nitrogen in the seeds. Tomato stem cuttings may be produced which contain thirty-six parts of carbohydrates (starch, sugar, free-reducing substances) to one of nitrogen; whereas the starchy wheat grains have only about seven parts of carbohydrates to one of nitrogen. The carbohydrates may thus tend to become limiting factors in the growth of seedlings in darkness more quickly than they did in the growth of cuttings.

2. Is fertilization with nitrogen advisable in the early growth of all types of seedlings, regardless of weather and light conditions and the nitrogen reserves of the seed?

The results obtained in some experiments with seedlings grown at different seasons of the year show that, with the exception of rice seedlings, all the types can assimilate nitrate nitrogen into growth-promoting substances in the early phases of growth, if light conditions are favorable and permit of rapid and abundant synthesis of carbohydrates. On the other hand, if conditions are unfavorable for the synthesis of carbohydrates, the very high-nitrogen types of seedlings may grow as well without as with nitrates in their early growth. Nitrates become beneficial after the seedlings have developed a photosynthesizing surface. The rate of nitrogen assimilation is closely correlated with the rate of synthesis of carbon compounds. This sort of response also agrees to some extent with results found with tomato cuttings. There it was noted that nitrates were toxic to cuttings having a very high-nitrogen but low-carbohydrate content when kept in darkness, and that nitrates were of no directly noticeable benefit in the light until more carbohydrates had accumulated by photosynthesis.

3. Is the seedling with the larger reserve of carbon better able to live and thrive during prolonged periods of dull, cloudy weather during the early growth of the plant?

4. Is the seedling with the larger amount of stored nitrogen at its disposal better able to withstand conditions of drought or of poor soil during the early phase of development when nitrogen from an outside source is unavailable or available in very limited amounts?

There is some evidence that those seedlings endowed by the parent plant with an abundance of readily available carbon com-

pounds will make the most rapid growth at first. This is true, not only of those with a limited nitrogen supply, but also of those grown from higher-nitrogen, high-fat seeds in which there is a great abundance of sugar produced by a rapid hydrolysis of the fats. Sunflower and tomato seedlings have this mode of behavior. These two types of seedlings and those of the grass family grew very rapidly during the first week. The results suggest that seedlings having an abundance of readily obtainable carbon reserves are better able to grow during cloudy weather in the early stages of growth. On the other hand, the seedlings having the larger stores of nitrogen are better able to grow and establish themselves in nitrogen-poor soil. They will tend to become deep-rooted under such conditions, and should be able to absorb a greater amount of nitrogen from the substrate because of the wider spread of roots. A period of abundant nitrogen and relatively small carbon supply in the early stages of growth tends to make leafy but shallow-rooted plants, which may not be so well adapted to obtain their needed amount of nitrogen and other minerals in their later development.

KOSINSKI (7), who was probably the first to study this relation of nitrogen to the growth of different organs, found that nitrogen feeding restricts the growth of roots in length and favors that of the stem. GODLEWSKI, in whose laboratory part of KOSINSKI's experiments were conducted (the work was begun in the botanical laboratory at Jena), incidentally utilized some of his barley seedlings for proof of KOSINSKI's results. His observations (6) agreed with those of KOSINSKI, and showed besides that the presence of sugar favored the growth of roots in length. He states that:

root growth was especially favored in comparison with shoot growth if the nitrogen-free solution contained sugar, probably because in the more abundant supply of carbohydrate material, the nitrogen-hunger was accentuated. It seems as if the plants, hungering for nitrogen, strive through the appropriation of the largest possible quantity of the materials available for growth of roots to make better use of the scanty nitrogen supply in the soil.

In the same manner as GODLEWSKI has attempted to account for the favoring of growth of roots by a limited supply of nitrogen and an abundance of carbohydrates, one might explain the stimulation in growth of shoots (particularly of leaves that results when

nitrogen is abundant) as an effort on the part of the plant to expose a great amount of surface to the light so as better to provide for an increase in the products of photosynthesis. It seems more probable, however, that the differences in growth are due more directly to differences in the chemical conditions within the tissues rather than that they develop as purposeful adaptations.

GODLEWSKI (5) observed that:

the roots of etiolated plants are usually shorter than those of plants grown in the light, yet the difference is so small that on no account can the greater length of the hypocotyl of (plants grown in darkness) be compensated by the decrease in length of the roots.

The effect of light on the growth of roots has been neglected by most of the investigators who have studied the characteristics of the etiolated plant in contrast with those of the plant grown in the light. Perhaps the most conspicuous feature of the results reported here is that of the stimulating effect of light on root development. The light-favoring effect on growth of roots is most pronounced in the case of seedlings grown from high-protein seeds. Some of the low-protein types, as low-protein corn, produce root systems as large in darkness as in light when nitrogen is lacking in the nutrient medium. A similar response was noted with tomato cuttings. If they had a very large supply of carbohydrates and were not given extra nitrogen, roots grew almost as well in darkness as in light. It thus seems probable that the light-favoring effect on the growth of roots is directly or indirectly connected with the synthesis of carbohydrates.

These experiments have also demonstrated the well known fact that light has a limiting influence on growth in length of stems. Whether the growth-inhibiting effect is due indirectly to the synthesis and accumulation of carbohydrates, or to its effect on nitrogen and other forms of mineral metabolism, or to more directly stimulating effects on the protoplasm itself, has long been a much discussed question, and one for which a definite answer is still lacking. If seedlings having a supply of reserve carbohydrates are grown in light in an atmosphere lacking carbon dioxide, growth of the stem may equal that of a plant kept in the normal atmosphere under conditions that permit the rapid synthesis of carbohydrates. From this it has been concluded that the growth-limiting effects of light

on the stem cannot be directly due to the synthesis of carbohydrates. GODLEWSKI'S (5) experiments have shown that a greater quantity of dry matter may pass from the cotyledons into the hypocotyl, if seedlings are grown in darkness than if grown in light in an atmosphere lacking carbon dioxide. He found that stems of 14-day old *Phaseolus* seedlings grown from seeds of the same weight contained 202 mg. of dry matter when grown in darkness, and 141 mg. of dry matter when grown in light in air lacking CO<sub>2</sub>. It may be concluded that the hypocotyls of etiolated plants are longer than those of plants grown in the light, partly because more of the food reserves are used in their growth, and also because they have a considerably higher water content.

GODLEWSKI (5) also sought to determine whether the growth-limiting effects of light on the hypocotyl were exerted directly on it, or more indirectly through its influence in favoring the growth of the cotyledons. Using black paper cases, he darkened the cotyledons only of some etiolated *Raphanus* seedlings and the hypocotyls only in others. Similar seedlings were left uncovered and set out in normal atmosphere. He found that darkening the hypocotyl only caused a definite increase in its length; secondary to this, the cotyledons of the plants with the darkened hypocotyls were somewhat smaller than those of plants grown wholly in the light. Darkening the cotyledons only had a strong influence on their growth; they were larger than the cotyledons of completely etiolated plants. He stated, however, that the cotyledons became somewhat green at the base. It may be possible that the traces of light, which undoubtedly must have entered, caused a stimulation in growth of the cotyledons. BATALIN (1) presented some experimental evidence which showed that illumination of short duration exerts a remarkable influence on the growth of leaves. GODLEWSKI also has shown that illumination for a brief period favors the growth of cotyledons of *Raphanus* seedlings. Later experiments of TRUMPF (15) have demonstrated similar results.

The effects of light on the growth and development of different organs of seedlings will be discussed more fully in two following papers. Additional data will also be presented which help somewhat to indicate to what extent the influence of light on the growth of



different organs is due to the processes of assimilation and utilization of carbohydrates, and to what extent to the influence of light on metabolism and growth independently of carbohydrate synthesis.

#### SUMMARY OF RESULTS OBTAINED WITH SEEDLINGS GROWN IN LIGHT

1. Seedlings developed from seeds with high-nitrogen content, when grown with no external source of nitrogen, have a greater weight of shoots in proportion to that of roots than those which develop from seeds with low-nitrogen content.

2. The weights of shoots in proportion to roots of seedlings grown in the light is lower than those of seedlings grown in darkness. The shift in the proportions is brought about especially by the shorter stems, and in most cases by much larger root systems of the seedlings grown in the light.

3. The difference in the effect of light and darkness upon the weight of tops in proportion to roots is greater with seedlings from high-protein seeds grown without extra nitrogen than with seedlings grown from low-protein seeds.

4. Light does not greatly favor growth of seedlings from low-protein starchy seeds unless extra nitrogen is supplied. The leaves of these plants are considerably smaller and even weigh less in the light. The roots of some of these seedlings gain considerably in the light, however.

5. Light favors the assimilation of nitrates, especially by high-protein seedlings with relatively low carbon reserves. The assimilation of nitrates and utilization of the synthesized products favor the growth of shoots more than of roots.

6. When the nitrogen supply is limited to the reserves in the seed, the shoot to root ratios tend to diminish after the first week, but if nitrogen is abundantly supplied, the ratio tends to increase continuously during the first three weeks of growth of seedlings in the light.

7. The relative proportions of shoots to roots vary with the season of the year. In the long-day months the ratio is relatively low and in the short-day months it is higher.

8. Light favors the process of secondary thickening in the roots

and stems of dicotyledons, and of thickening of cell walls in all types of seedlings.

### General summary

1. Growth of the seedling is influenced by the nature and relative amounts of the food reserves of the seed, as well as by differences in the external environment such as light and darkness, and the presence and absence of nitrates in the nutrient solution. When the seedlings are grown without nitrogen from an outside source the following responses have been found: (a) Seeds having a high-nitrogen and relatively low-carbon content produce seedlings with a large top in proportion to the roots. (b) Seeds having a low-nitrogen and high-carbon content produce seedlings with a relatively small top in proportion to the size and weight of the roots. (c) Seeds intermediate in the proportions of their reserves of carbon and nitrogen produce seedlings with intermediate proportions of shoots to roots.

The following table illustrates the nature of the results:

TABLE XII  
WEIGHT IN GRAMS OF SHOOTS AND ROOTS PER PLANT

	RESERVE FOODS	LIGHT			DARKNESS		
		Stem peti- oles	Leaves	Roots	Stems	Leaves	Roots
High-protein corn...	Moderately high nitrogen	0.634	0.838	1.540	0.774	0.696	0.686
	Moderately high starch						
Low-protein corn...	Low nitrogen	0.298	0.336	0.800	0.715	0.575	0.750
	High starch						
White Marrow beans	Moderately high nitrogen	1.264	1.754	1.544	1.882	0.111	0.526
	Moderately high starch						
Cow peas.....	High nitrogen	0.677	0.420	0.906	0.699	0.058	0.237
	Moderately low starch						
Sunflower.....	High nitrogen	0.643	0.607	0.757	0.855	0.165	0.105
	High oil						
Muskmelon.....	Very high nitrogen	0.150	0.221	0.303	0.211	0.040	0.040
	High oil						

2. Nitrates are synthesized into growth-promoting substances, both in light and in darkness, but much more rapidly in the light.

3. Nitrates favor the growth of shoots more than of roots.
4. Light strongly favors the growth of roots.
5. (a) Seedlings developed from high-protein seeds benefit most under the influence of light. The roots and leaves are larger, more numerous, and much heavier than in the case of seedlings grown in darkness. This applies to high-protein seedlings grown with and without extra nitrogen, but the effect is greater in the case of the latter. (b) Seedlings grown from low-protein seeds without extra nitrogen are influenced less by light as to weight of different organs. Leaves of the very low-protein types grow even less in light than in darkness. When extra nitrogen is supplied these seedlings also benefit by the influence of light.
6. Seedlings with limited nitrogen supply undergo rapid differentiation and maturing of tissues in the light. The lower the nitrogen content of the seed the more rapid the process.
7. Light favors secondary thickening in stems and roots and deposition of strengthening materials in the cell walls.
8. The responses as to the effect of varying amounts of reserve carbon and nitrogen on growth of the seedling agree with results obtained with tomato cuttings having similar (although in some cases more extreme) variations in composition of the reserves.

These investigations were conducted at Boyce Thompson Institute for Plant Research, in 1924-1926. I wish to express my appreciation to the Cereals Division of the United States Department of Agriculture for the samples of pure line Marquis wheat and Hännchen barley used in these experiments; also to the Department of Plant Genetics of the University of Illinois for the seeds of high- and low-protein corn.

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## EXPLANATION OF PLATES I-IV

### PLATE I

Seedlings grown in darkness without nitrates:

FIG. 1.—Soy bean.

FIG. 2.—Cow pea.

FIG. 3.—Muskmelon.

FIG. 4.—Tomato.

FIG. 5.—Sunflower.

FIG. 6.—Illinois low-protein corn.

FIG. 7.—Illinois high-protein corn.

### PLATE II

Seedlings grown in light without nitrates:

FIG. 8.—Soy bean.

FIG. 9.—Cow pea.

FIG. 10.—Muskmelon.

FIG. 11.—Sunflower.

FIG. 12.—Illinois low-protein corn.

FIG. 13.—Illinois high-protein corn.

*PLATE III*

Tomato seedlings grown in light:

FIGS. 14, 16, 18, and 20.—In nutrient medium lacking nitrogen at stages of growth at one, two, three, and four weeks respectively.

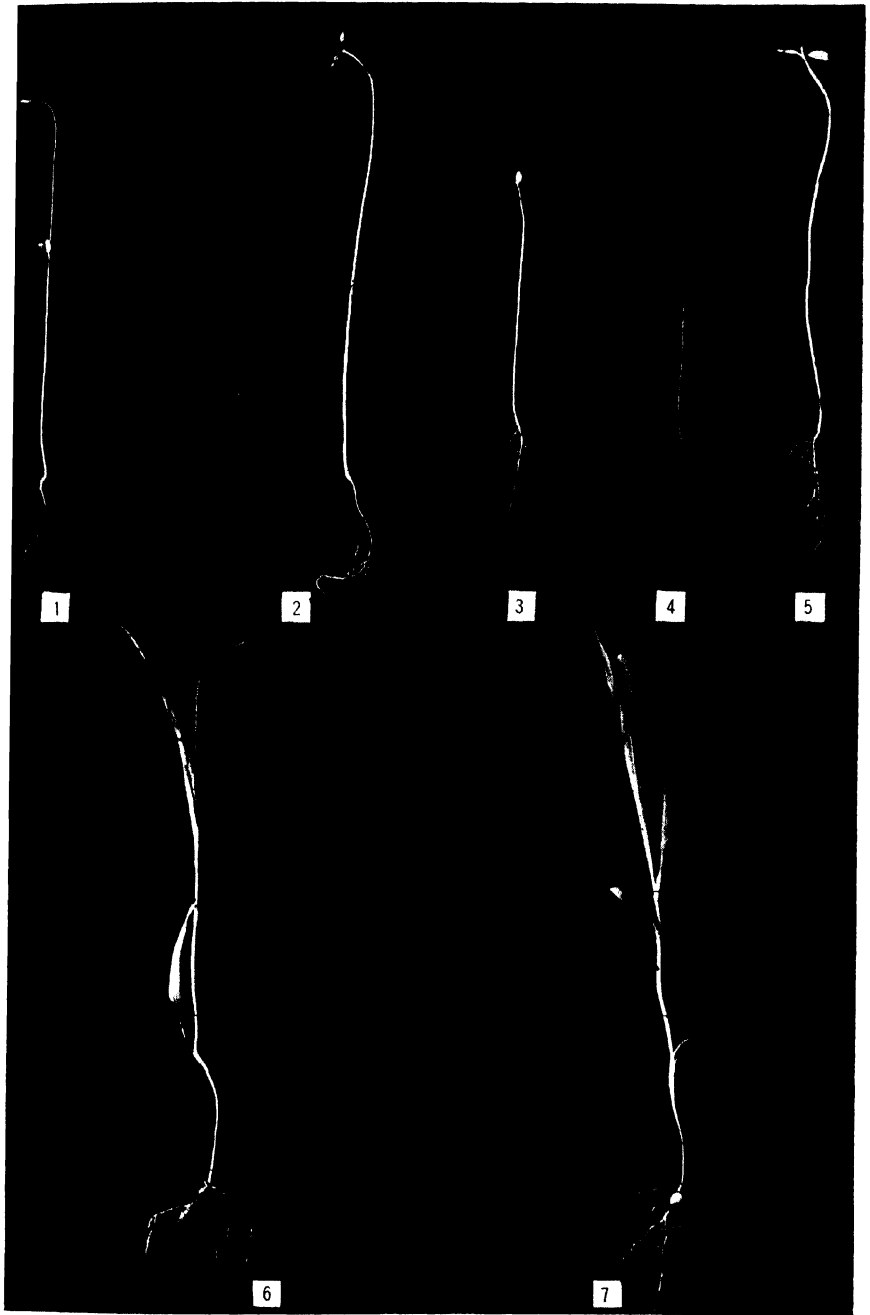
FIGS. 15, 17, 19, and 21.—In nutrient medium containing nitrates at stages of growth of one, two, three, and four weeks respectively.

*PLATE IV*

Sunflower seedlings grown in light:

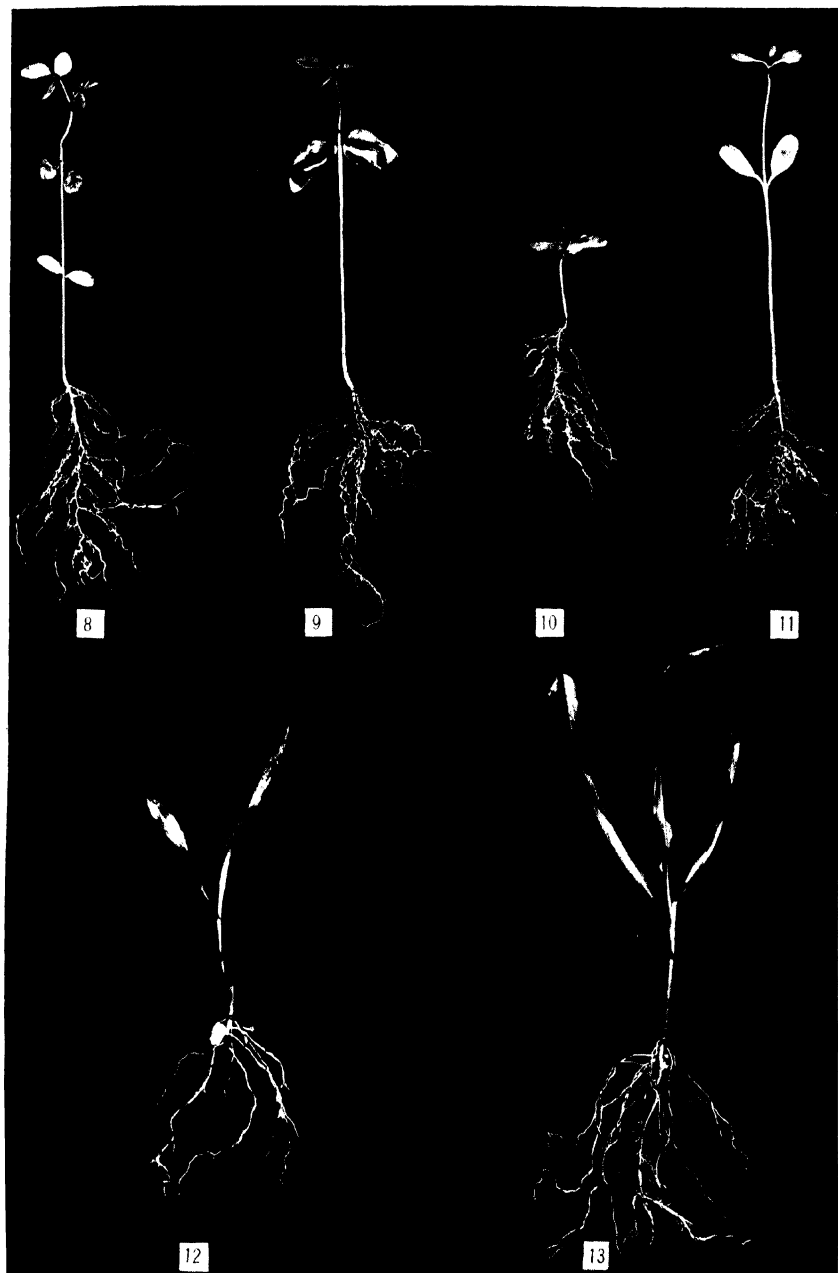
FIGS. 22, 24, 26, and 28.—In nutrient medium lacking nitrogen at stages of growth of one, two, three, and four weeks respectively.

FIGS. 23, 25, 27, and 29.—In nutrient medium containing nitrates at stages of growth of one, two, three, and four weeks respectively.



REID on SEEDLING GROWTH

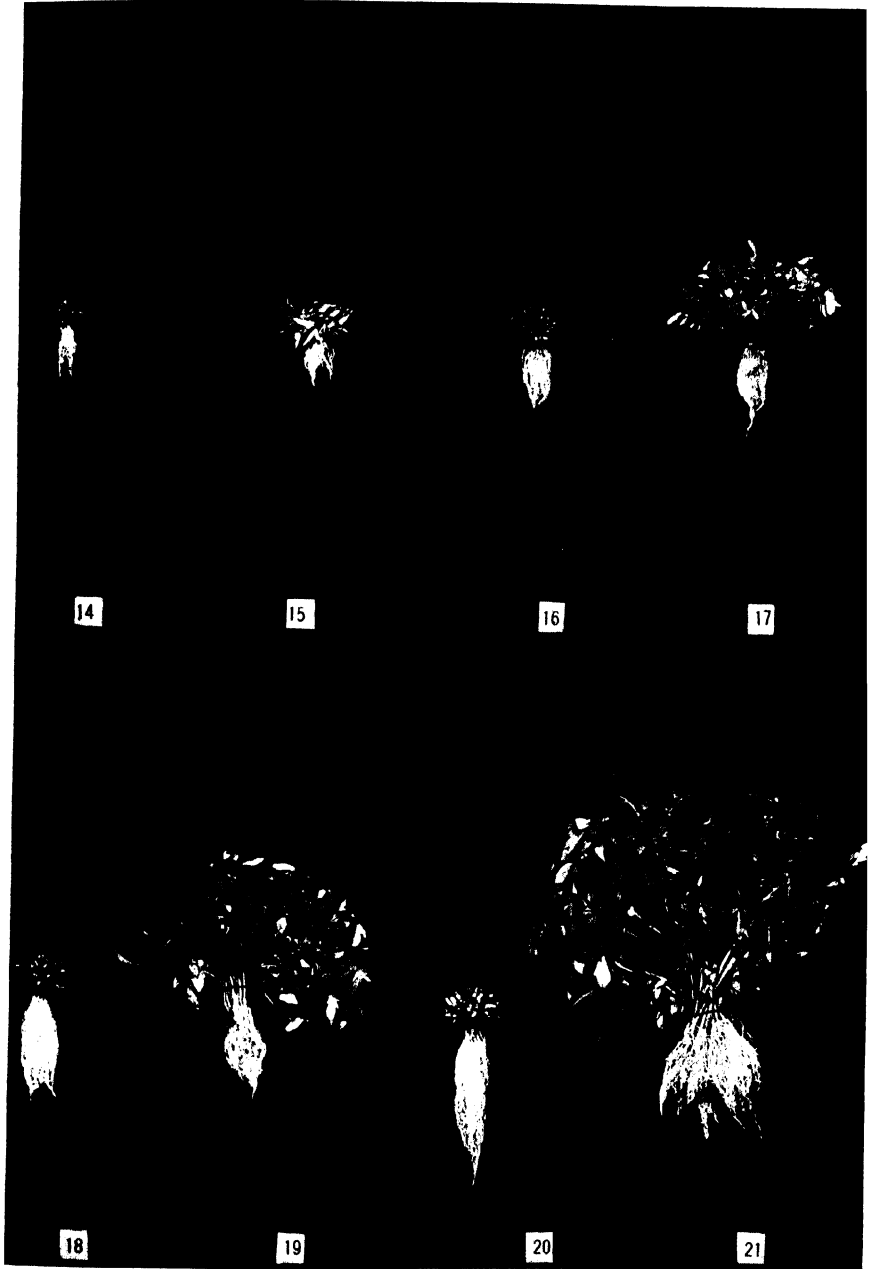




REID on SEEDLING GROWTH

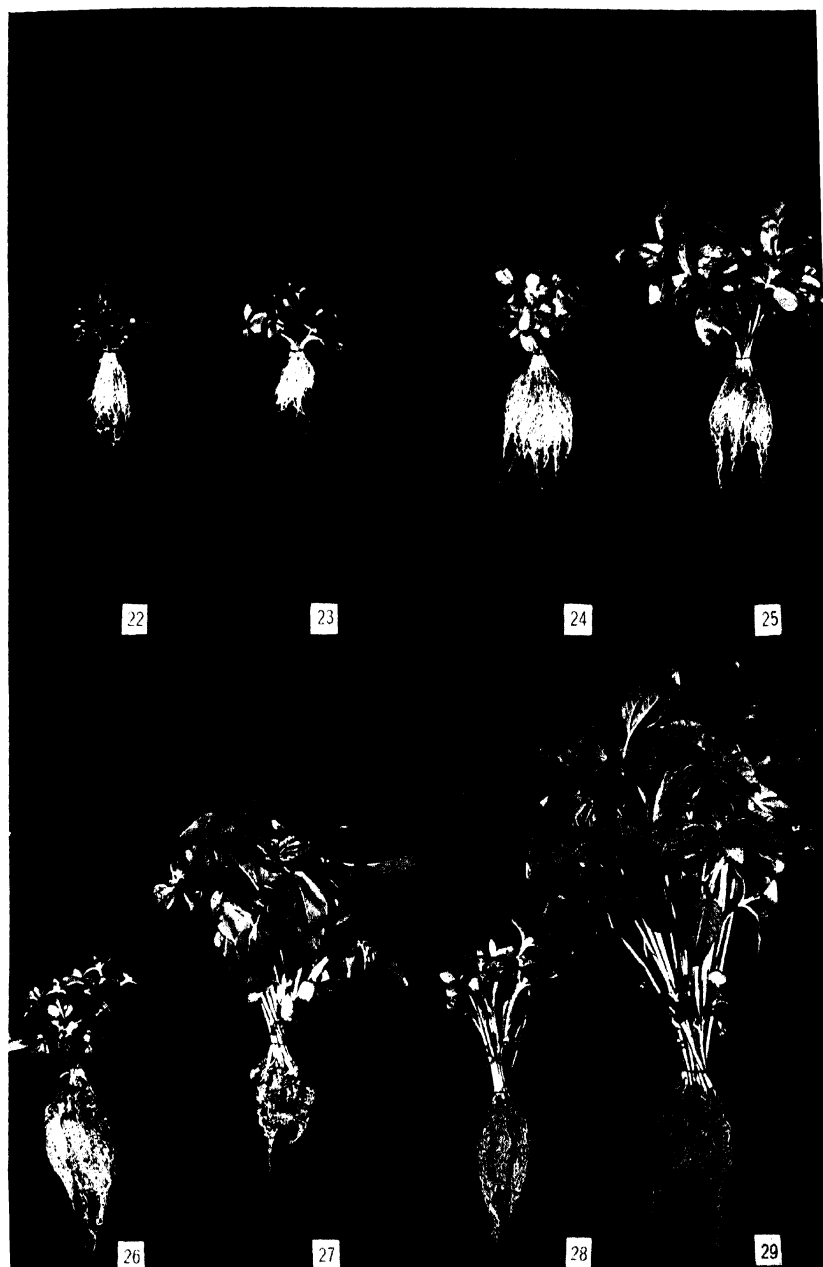






REID on SEEDLING GROWTH





REID on SEEDLING GROWTH



## ORIGIN OF ADVENTITIOUS ROOTS IN COLEUS CUTTINGS<sup>1</sup>

MARGERY C. CARLSON

(WITH PLATES V, VI)

### Introduction

In connection with some microchemical work on the rooting of *Coleus* cuttings, it was necessary to know the origin of their adventitious roots. The extensive work of LEMAIRE (1) was concerned with the origin of naturally occurring endogenous adventitious roots in the hypocotyls, stolons, and rhizomes of many herbaceous dicotyledons. He grouped the plants studied into the following four classes, of which the first is most common: (1) all tissues of the root originating in the pericycle of the stem; (2) the central cylinder of the root coming from the pericycle, and the other regions from the endodermis and inner cortex of the stem; (3) all tissues of the root coming from the "subphloem meristem" (cambium); (4) the central cylinder of the root formed by the cambium, and the other tissues by the pericycle of the stem.

VAN TIEGHEM and DOULIOT (5) added to their investigation of the origin of secondary roots a reinvestigation of the problem of the origin of natural adventitious roots in stems, chiefly hypocotyls and rhizomes. They came to the conclusion that endogenous roots arise entirely from the pericycle, except in older portions of stems where the pericycle has lost its "root-forming character." They define the pericycle as the layer, or layers, between the endodermis and the external phloem of a fibrovascular bundle, continuous across the medullary rays, but not distinguished on the inside from the medullary ray.

If the pericycle is simple, an arc of cells elongates radially and divides by tangential walls. The internal layer becomes the central cylinder of the new root. A tangential division of the external layer

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

separates the cortex (internal) from the epidermis (external). The cells of the epidermal layer surrounding the tip of the root divide again to form the root cap. If the pericycle is compound, it is usually its external layer which produces the epidermis, cortex, and central cylinder of the root. The other layers produce only the internal region of the central cylinder. When the root arises after the pericycle has lost its "root-forming" property, it may originate in the external primary phloem parenchyma, the internal primary phloem parenchyma, or the secondary phloem parenchyma.

Some recent work has dealt with the origin of adventitious roots in cuttings. SMITH (2), using cuttings of *Coleus blumei*, reports that "the first recognizable sign of the development of an adventitious root is the appearance in the cambium of a nest of highly meristematic cells." VAN DER LEK (4) described "root germs," present in young branches of *Ribes nigrum*, *Salix*, and *Populus*, as being "in connection with the cambium" as a "continuation of a medullary ray." SWINGLE (3) finds "root germs" initiated in the cambium ring of apple stems which produce burrknots.

### Materials and methods

The work here reported was restricted to a study of the origin and development of adventitious roots arising between the fibrovascular bundles in young stem cuttings of *Coleus blumei*.

The cuttings, 2-6 inches long, were made from the tips of branches of *Coleus* plants and placed with their cut ends in water or in sand. The cuts were usually made through the internodes. The basal portions of these cuttings were removed and preserved at intervals beginning with the third day. These pieces of stems were fixed in formalin-acetic-alcohol and imbedded in paraffin. Sections, both transverse and longitudinal, were cut 12-15  $\mu$  in thickness and stained with safranin and gentian violet. In all cases serial sections were studied, and the median section of the primordium or young root was used for the figures.

In this paper the terms "origin" or "initial" will be used to indicate the cell, or group of cells, which by division initiate an adventitious root; "root primordium" to mean the group of meristematic cells from the time of earliest divisions of the "origin" to the

time of differentiation into well marked regions characterizing a young root.

### Observations

The structure of the stem of *C. blumei* has been described by SMITH (2). The arrangement and differentiation of the tissues in the stems used in this work are shown in figs. 1, 3, and 5. The outer cortex at the stages shown has not yet developed into collenchyma. The endodermis is a somewhat regular layer of cells, usually smaller than the typical cortical parenchyma cells, and is easily detected because of the presence of large, compound starch grains (fig. 4). The pericycle consists of a single layer of cells just inside the endodermis. In stems older than those used for this study the pericycle opposite the vascular bundles differentiates into sclerenchyma.

Four large fibrovascular bundles occupy the angles of the stem. One or two smaller bundles lie between each two contiguous corner bundles (fig. 7). Isolated groups of primary phloem cells occur between the bundles. Such groups are seen in figs. 1 and 3. The layer of pericycle cells lies just outside these groups of cells.

A continuous cylinder of cambium was well developed in all the stems studied. In some cases the cambial cells had just begun to divide to form secondary xylem and phloem (fig. 7), but more commonly three to five layers of secondary xylem and one or two layers of secondary phloem were already present (figs. 1, 3, and 5). The interfascicular cambium arises one or two layers inside of the pericycle. There are, therefore, one or two layers of parenchymatous cells between the pericycle and the interfascicular cambium.

SMITH (2) finds that "the first roots appear in four ranks, corresponding to the four vascular strands. This arrangement may be obscured later by roots which arise irregularly in between." As a rule, the findings reported in this paper are in agreement with SMITH's observations.

Table I shows the age and position of root primordia and adventitious roots in seven cuttings chosen at random. The roots are numbered in the order of their appearance. The oldest root, that is, the first to appear, is numbered 1; the next younger, 2, etc. The numbers are arranged in columns with respect to the position of the roots in the stem from the cut surface upward; the lowest number



in a column therefore represents the lowest root in the stem, and so on. The letters following the numbers indicate the position of the adventitious roots with respect to the fibrovascular bundles. "C" indicates that the root appears opposite a large corner bundle; "EC," at the edge of a corner bundle; "L," opposite a lateral bundle; "B," between two bundles.

TABLE I  
ORDER OF APPEARANCE AND POSITION OF ROOTS AND ROOT  
PRIMORDIA IN SEVEN COLEUS CUTTINGS  
(EXPLANATION IN TEXT)

CUTTING NUMBER						
I	II	III	IV	V	VI	VII
1-C	1-C	3-EC	1-C	1-C	1-B	3-C
2-C	5-C	2-EC	2-C	5-EC	6-L	5-B
3-L	3-B	1-C	3-C	2-B	6-B	2-EC
4-C	2-C	4-EC	1-C	3-C	7-L	3-C
6-EC	4-C	1-C	4-B	4-C	2-C	1-C
5-C					7-L	3-C
					2-C	6-B
					4-C	7-B
					8-B	6-B
					5-EC	5-B
					3-C	4-EC
						5-B
						10-B
						6-B
						10-B
						6-B
						10-B
						7-B
						8-B
						9-B
						10-B

In general, the first four or five roots arose opposite corner bundles, while later roots arose opposite lateral bundles or between them. An exception was found in four of the cuttings examined. In cutting VI the first root arose between the bundles; in cutting V, the second; in cutting III, the third; and in cutting IV, the fourth.

There was no regularity in the appearance of the roots with respect to the cut surface. Sometimes the first roots arose nearest the cut surface (table I, cuttings III and IV), and sometimes farthest from the cut surface (cuttings I, II, IV, V, VI, and VII). All roots appeared within a distance of 2-3 mm. from the base of the cuttings.

The first evidence of adventitious root formation is an accumulation of protoplasm and an increase in the size of the nucleus and of the nucleole in one cell or in several adjacent cells of the pericycle. The enlarged nucleus takes a central position. The cell or cells then divide.

Fig. 1 shows three neighboring pericyclic cells which have divided, thus initiating an adventitious root. The cell on the left divided tangentially, and then the inner daughter cell divided tangentially. The middle cell of the group divided by a tangential wall; its inner daughter cell divided by a tangential wall and then the outer granddaughter cell divided slightly obliquely. The first division of the cell on the right of the group was also tangential.

In fig. 2 a pericyclic cell has divided either tangentially or radially, and its daughter cells either radially or tangentially. The cell on its left has divided into two very unequal daughter cells by an oblique wall in the upper corner. Several adjoining pericyclic cells on the right have each divided tangentially.

From a study of serial sections of the primordium shown in fig. 3, it was evident that the first division of the initial pericyclic cell was radial. Each daughter cell then divided tangentially into two unequal cells, the inner one being larger than the outer. The inner left cell must have divided transversely and one of its daughter cells then radially, the other obliquely. Only the cell with the oblique wall shows in the figure. Divisions had proceeded further in the two outer granddaughter cells. The one on the right divided transversely and its upper daughter cell radially into two unequal cells.

In other cases studied the division of the cells of the root origin was radial, or occasionally oblique, but usually the division was tangential. The divisions of the daughter cells may be tangential, transverse, or radial. LEMAIRE (1) and VAN TIEGHEM and DOULIOT (5) found that the initial cells of a primordium always divided tangentially, and that the external layer of daughter cells always divided again tangentially, thereby making three layers of cells which developed into the three regions of the young root. In the work herewith reported no such regularity in development was found.

Cell divisions continue without much enlargement of the daughter cells before their divisions, until the space occupied by the original

one (or several) active pericyclic cells is filled by a large number of small, more or less cubical, meristematic cells (figs. 5-7).

Pericyclic cells adjacent to the initial and parenchymatous cells toward the inside of the initials (fig. 5) begin to divide and become a part of the root primordium. The history of the divisions of these cells is similar to that of the initial cells. In fig. 11, for example, the cell on the extreme left of the primordium has divided either tangentially and its daughter cells radially, or radially and its daughter cells tangentially. The cell lying next to it toward the right has divided tangentially; its daughter cells have divided tangentially. The two inner granddaughter cells divided radially. The cell to the right of the median cell in the primordium shown in fig. 12 has probably divided first radially, then each of its daughter cells tangentially, and each of the granddaughter cells again tangentially, forming two regular radial rows of four cells each.

Further development of the primordium consists in continued cell divisions, enlargement of the cells, and the addition of other neighboring cells (figs. 8-12). It is impossible to trace the order of cell divisions in the older primordia. The cells which resulted from the division of the median initial cell begin first to enlarge (fig. 9). The cells from adjacent initial cells follow, and as enlargement of the cells proceeds the primordium bulges toward the outside (figs. 9-12). The cells of the endodermis divide radially to allow for the push from the developing primordium (figs. 9, 12).

Figs. 7 and 8 show the position of the root primordia in relation to the lateral fibrovascular bundles. The pericycle can be followed outside the primary phloem of the bundle. The interfascicular cambium is continuous with the cambium of the bundle. It is evident that the cambium has taken no part in the formation of the primordium. Several rows of undivided cells lie between the young primordium and the cambium.

The position of the young primordia in relation to the groups of primary phloem cells is best seen in fig. 3.

There is no evidence of differentiation into the epidermis, root cap, cortex, and central cylinder of a root in any of the stages of development previous to that shown in fig. 12. All of the cells of the primordium seem to be equally capable of division. Several nuclei in process of division can be seen in fig. 12.

Fig. 13 shows a root primordium which has become definitely hemispherical and has begun to differentiate into the tissues of a root. The single external layer of cells becomes the epidermis. Two or three layers of cells inside the epidermis become the cortex and the innermost region becomes the central cylinder. A slightly older root (fig. 14) shows the beginning of the root cap from the tangential division of the epidermal cells which surround the apex.

The further development of the root has been adequately described by former investigators. Elongation proceeds by the division of cells in all parts of the young root, and by elongation of the cells of the central cylinder. The endodermis incloses the developing root for a short time, then breaks or dissolves. The cavity opposite the apex of the root (figs. 13, 14) indicates that the cells of the cortex are being dissolved before the growing root. Finally the tissues of the central cylinder differentiate, connection is made with the vascular system of the stem, and the root emerges.

### Summary

1. Adventitious roots arising between the fibrovascular bundles from the bases of young cuttings of *Coleus blumei* originate in one to several adjacent cells of the pericycle.

2. The first division of the initial cells of a root is usually tangential, but it may be radial, or even oblique.

3. During the early stages of development the daughter cells divide without first increasing in size, so that a young primordium consisting of many cells may occupy the same space as the pericyclic cells from which it originated.

4. The primordium enlarges and bulges into the cortex before differentiating into the tissues of a root.

5. Subsequent development is precisely as described by the early investigators of the subject of root development.

The writer wishes to express her appreciation to Dr. C. E. ALLEN, Department of Botany, University of Wisconsin, Madison, Wisconsin, for criticisms on the manuscript while in preparation.

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# EXPLANATION OF PLATES V, VI

All figures are photomicrographs. The walls of the cells and the nuclei were traced with India ink, and the photographs then bleached. The plates were reduced one-fourth. Figs. 1-12 are transverse sections of stem cuttings; figs. 13 and 14 are longitudinal sections of stem cuttings. All sections of root primordia except fig. 11 are median.

## PLATE V

FIG. 1.—Transverse section of stem, showing very young root primordium: *e*, epidermis; *c*, cortex; *en*, endodermis; *p*, pericycle; *pph*, primary phloem; *sph*, secondary phloem; *ca*, cambium; *sx*, secondary xylem; *pi*, pith.

FIG. 2.—Young root primordium.

FIG. 3.—Young root primordium, showing its relation to group of primary phloem cells.

FIG. 4.—Young root primordium; starch grains in endodermis.

FIG. 5.—Young root primordium, showing adjacent pericyclic cells and parenchymatous cells beginning to divide.

FIG. 6.—Root primordium, later stage than fig. 5.

FIG. 7.—Root primordium, showing its relation to lateral fibrovascular bundle.

## PLATE VI

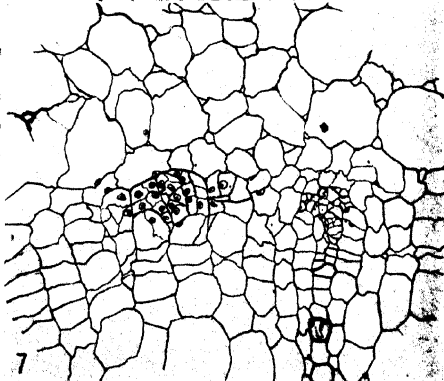
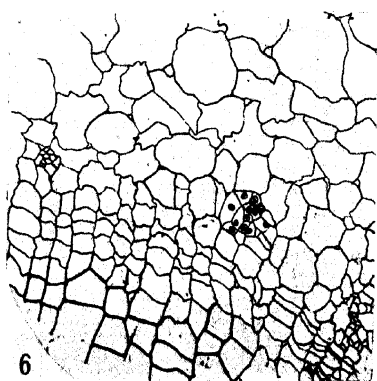
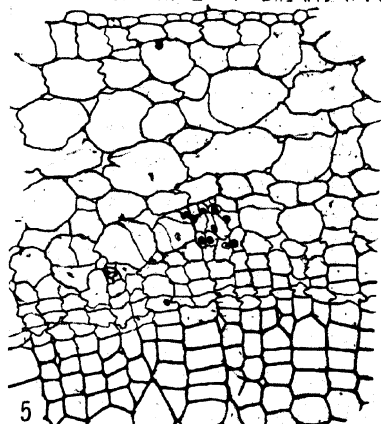
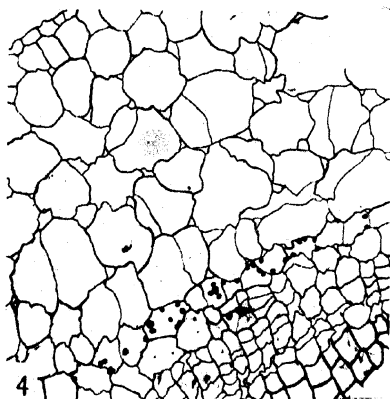
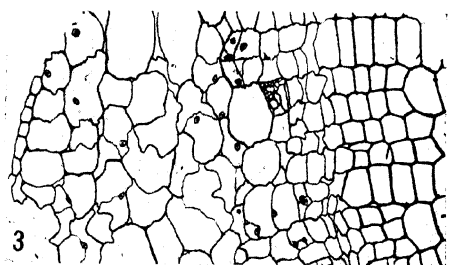
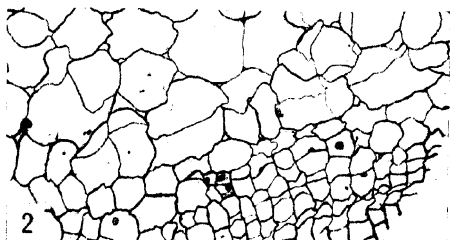
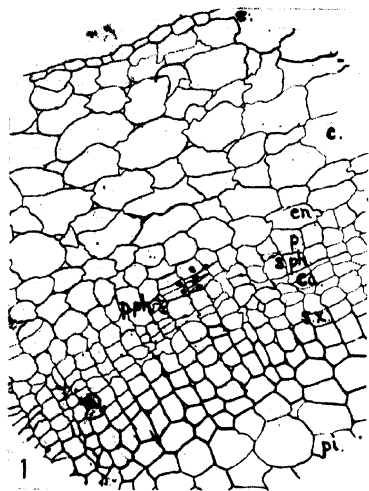
FIG. 8.—Root primordium, consisting of many cells, but occupying little more space than cells from which it originated.

FIG. 9.—Root primordium, beginning to bulge into cortex; endodermal cells bordering primordium have divided; cambium has taken no part in production of primordium.

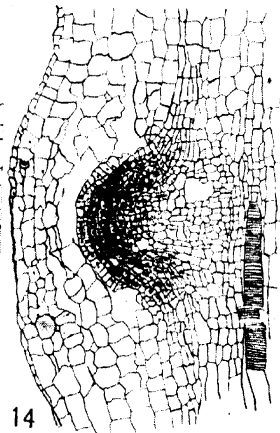
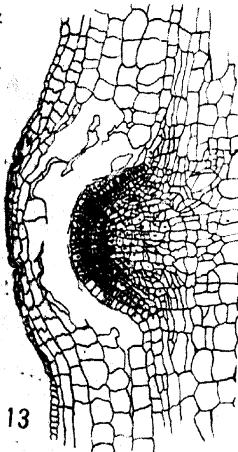
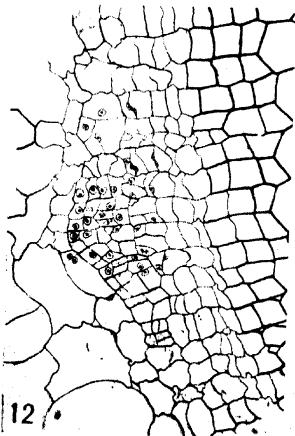
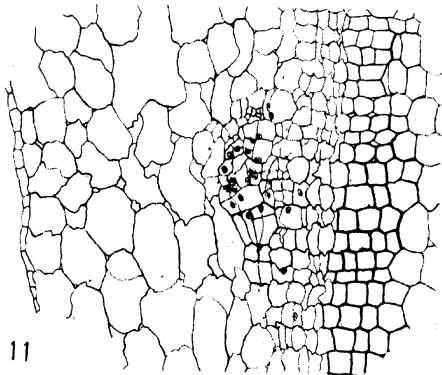
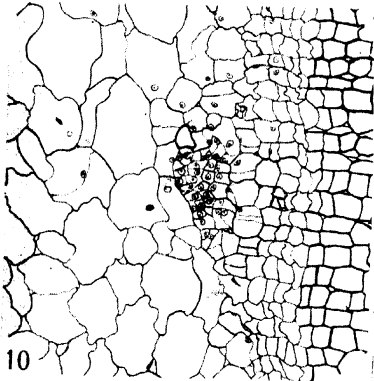
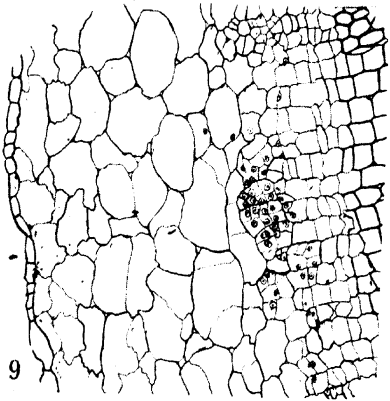
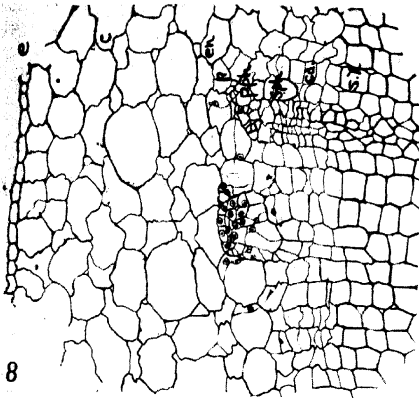
FIGS. 10-12.—Older primordia.

FIG. 13.—Young root in which differentiation into epidermis, cortex, and central cylinder has begun; endodermis and part of cortex dissolved.

FIG. 14.—Young root, later stage than fig. 13; root cap beginning to form.











## GERMINATION AND VITALITY OF BIRCH SEEDS<sup>1</sup>

HILDA C. JOSEPH

(WITH FIVE FIGURES)

### Introduction

In a recent paper, WEISS (13) has given his results with experiments on temperature and medium requirements for the germination of fresh and after-ripened seeds of *Betula populifolia*. He finds that the germination of *B. populifolia* is greatly improved when the seeds are stored in moist granulated peat at low temperature for about two months; that 10° C. is as effective as 5° C. or 0° C. for this purpose; that germination percentage is increased by a treatment with an organic mercury disinfectant previous to stratification; and that such after-ripening at low temperatures results in a marked downward shift in the minimum temperature required for germination.

In the work reported in this paper, the writer has extended studies of the same type to other species of *Betula*, and has also tried to determine whether seeds of the same species vary in their behavior when collected at different times of the season and when kept in different conditions of storage for a year or more.

### Material and methods

The main studies have been with *Betula lenta* seeds, which were collected from trees in the forest of the Boyce Thompson Institute for Plant Research. September, October, and November collections were made from the same trees. Since the seeds of the October collection were superior to all others, they were used exclusively in the storage experiments.

The seeds of *Betula populifolia* used in these experiments were also collected from the Institute's forest, while *B. papyrifera* and *B. lutea* were commercial seeds, harvested and shipped by seed deal-

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

ers in October, 1926. All seeds were freed from wings by treatment in a Hobart mixer before being used.

In making the germination tests, Petri dishes with three layers of filter paper were employed, except when another substratum is indicated. Two hundred seeds were placed in each dish.

In the early determinations on the optimum conditions for after-ripening, that is, for the improvement of germination through temporary moist storage at cool temperatures, the seeds were mixed with moist acid granulated peat, neutralized granulated peat, or sand, and placed in large-mouthed bottles covered with cheesecloth.

TABLE I  
CHANGE OF CONCENTRATION IN SOLUTIONS OF SULPHURIC ACID USED  
TO REGULATE MOISTURE CONTENT OF BETULA SEEDS

CONCENTRATION OF $H_2SO_4$ (PERCENTAGE)	SPECIFIC GRAVITY AT BEGINNING OF EXPERIMENTS	SPECIFIC GRAVITY OF FIRST SET OF SOLU- TIONS AT END OF FIRST 2 WEEKS	SPECIFIC GRAVITY OF SECOND SET OF SOLUTIONS AT END OF SECOND 2 WEEKS
Concentrated...	1.8300	1.8046	1.8181
64.8	1.5200	1.5050	1.5024
55.9	1.4300	1.4029	1.4185
43.4	1.3300	1.3042	1.3200
30.4	1.2234	1.2021	1.2092
18.4	1.1210	1.0334	1.1890

In the later studies the seeds were after-ripened in lots of 200 on filter papers in Petri dishes. This latter method proved just as effective and more practical for these studies, since the after-ripened seeds could then be used in germination tests without previous transfer from the bottles to Petri dishes.

The moisture determinations were made by drying non-macerated seeds to constant weight at  $103^\circ C$ . When seeds were prepared for dry storage at various moisture contents, the samples were brought to water vapor equilibrium with a given concentration of sulphuric acid in a desiccator, which was provided with a stirrer for both the air and the sulphuric acid solution. The solutions were changed every two weeks. The changes in specific gravity of the sulphuric acid solutions that occurred during the first two periods are given in table I.

After eight weeks of storage over  $H_2SO_4$  solutions, the seeds had

reached water equilibrium with the solutions. At this time one series of samples was taken out to determine the moisture content of the different lots of seeds; a second series was taken out for the purpose of germination tests; the third and largest amount of material was placed in small bottles, sealed air-tight, and stored under the following conditions: at room temperature, in an ice chest at about  $8^{\circ}\text{C}.$ , and in a room varying in temperature from  $-15^{\circ}$  to  $-8^{\circ}\text{C}.$  A different method of storage was employed with seeds that had been exposed to humidified atmospheres over sulphuric acid solutions of concentrations lower than 55.9 per cent. Here the seeds were sus-

TABLE II  
WEIGHT AND MOISTURE CONTENT OF VARIOUS COLLECTIONS OF BETULA  
SEEDS AS DETERMINED IMMEDIATELY AFTER HARVEST OR AFTER  
ARRIVAL OF SHIPMENT

COLLECTION	WEIGHT OF. 1000 SEEDS AIR-DRY (GM.)	WEIGHT OF 1000 SEEDS DRIED TO CONSTANT WEIGHT (GM.)	MOISTURE CONTENT IN PERCENTAGE DRY WEIGHT
Betula lenta, collection A and B, September, 1926.....	0.7300	0.6730	7.54
Betula lenta, collection, October, 1926....	0.5471	0.5084	7.09
Betula lenta, collection, November, 1926...	0.4863	0.4570	6.04
Betula populifolia, collection, October, 1926	0.0920	0.0853	7.36
Betula papyrifera, commercial collection, 1926.....	0.2740	0.2502	8.70

pending in a wire basket from the cover of a tightly closed museum jar over the different solutions, and stored in this way at room temperature and in an ice chest. In the low temperature room all seeds were stored in tightly sealed bottles. The total number of seeds of the various species and collections used in these studies approximates one million.

### Experimental results

#### 1. EXPERIMENTS WITH NEW SEEDS

It is common practice among gardeners and nurserymen to keep freshly harvested seeds on drying racks for a short time before they are planted. This is done mainly to avoid molding and fermentation in seeds surrounded by a fleshy pulp; but there are also cases in which a period of drying is directly beneficial to the germination

quality of the seed material. Wheat and other grains, especially if they have ripened during a rainy season, are improved by a period of dry storage.

It seemed to be of interest to determine whether such an improvement of germination quality could also be obtained in *Betula* seeds, and whether it could be obtained in seeds picked at the beginning of the harvesting season as well as in those picked at the end. Table II shows that the percentage of hygroscopic moisture of the seeds decreases with the advancing season, and that the weight for 1000 seeds is lower in the later collections.

TABLE III  
GERMINATION OF FRESHLY HARVESTED BETULA LENTA SEEDS  
IMMEDIATELY AFTER HARVEST

DESCRIPTION OF MATERIAL	NO. USED IN EACH TEST	PERCENTAGE GERMINATION AT			
		15° C.	20° C.	25° C.	32° C.
Collection A, September, 1926, from green catkins.....	500	0	5	3	7
Collection B, September, 1926, from brown catkins.....	500	0	2.5	5	3.5
Collection, October, 1926.....	200	0	0	0	17
Collection, November, 1926.....	200	0	0	1	2

The September collection was taken from closed catkins which were dried in the laboratory to induce shedding of the seeds. The October and November collections were taken from catkins that had already opened on the trees. The November collection had also been exposed to some freezing on the trees. The data in tables III and IV show that the germination quality is improved considerably in all the different collections through one month of dry storage at laboratory temperature. This improvement, therefore, must be due to changes in the seed different from those which are produced by nature through variations in humidity, temperature, and ventilation during the period in which the seed hangs on the tree.

Since acids have been found to have a favorable influence upon the germination of some seeds, it was thought possible that an acid

substratum for freshly harvested seeds might substitute for the beneficial effects of dry storage. Table V shows the negative results

TABLE IV  
GERMINATION OF BETULA SEEDS AFTER ONE MONTH OF  
DRY STORAGE IN LABORATORY

DESCRIPTION OF MATERIAL	NO. USED IN EACH TEST	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alterna- tion of 15°-32° C.
B. lenta, collection A, September, 1926.....	200	0	0	0	17	48
B. lenta, collection October, 1926..	400	0	0	0	41	46
B. lenta, collection November, 1926	200	0	0	0	11	39
B. papyrifera, commercial collec- tion 1926.....	600	0	9	67	80.5	81
B. populifolia, collection October, 1926.....	200	0	17	51	60	58
B. lutea, commercial collection, 1926	400	0	2	3	8	15

TABLE V  
INDIFFERENCE OF FRESH BETULA LENTA SEEDS TO ACIDITY OF SUBSTRATUM

DESCRIPTION OF MATERIAL	NO. USED IN EACH TEST	SUBSTRATUM	PERCENTAGE GERMINATION AT			
			15° C.	20° C.	25° C.	32° C.
Collection A, Septem- ber, 1926 from green catkins.....	500	Filter paper	0	0	0	4.6
	500	Peat pH 4.6	0	5.4	4	6
	500	Peat, neutral with excess CaCO <sub>3</sub>	0	1	0	5.8
Collection B, Septem- ber, 1926 from brown catkins.....	500	Filter paper	0	1	0	5
	500	Peat pH 4.6	0	3	5	3.5
	500	Peat, neutral with excess CaCO <sub>3</sub>	0	0	4	5
Collection October, 1926.....	200	Filter paper	0	0	1	17
	200	Peat, neutral with excess CaCO <sub>3</sub>	0	0	0	2

obtained with tests arranged for this purpose. Except for a probable slight improving of germination on acid peat at 20° C. of the earliest collection of *B. lenta*, there is no noticeable effect that could be interpreted as favorable influence of the organic acids of peat upon the germination quality of fresh seeds.

Similar tests were conducted to determine whether a favorable

effect produced by light, or by increased or reduced oxygen pressure, could be observed, but the seeds were not sensitive to considerable variations in intensity of these factors. A short time later the same experiments were repeated with laboratory-stored seeds with the same negative results.

A decrease of germination percentage occurred in laboratory-stored seeds of *B. lenta* after sterilization with 0.25 per cent uspulun, as shown in table VI.

Although laboratory-stored seeds germinate well at high temperatures, as 32° C. and at alternation of 15-32° C., they do not germi-

TABLE VI  
EFFECT OF STERILIZATION WITH USPULUN (0.25 PER CENT FOR ONE-HALF HOUR) UPON GERMINATION OF LABORATORY-STORED SEEDS OF *BETULA LENTA*; 200 SEEDS IN EACH TEST

DESCRIPTION OF MATERIAL	GERMINATION AT FAVORABLE TEMPERATURE			
	32° C.		Alternation of 15°-32° C.	
Collection October, 1926, sterilized...	68 63 68	Average 66.1	53 50	Average 51.5
Collection October, 1926, not sterilized.....	73 81 74	Average 76	58 59	Average 63.5

nate to a considerable percentage at constant temperatures below 32° C. By a suitable period of moist storage at cool temperatures this behavior can be changed. The seeds are "after-ripened" through this treatment, and afterward germinate better, not only at higher temperatures, but also at temperatures as low as 15° C. If kept in cool storage continuously they finally even germinate at 0° C. All species studied react in a similar way, although *B. papyrifera* is the least dormant of the species studied; and the improvement in total germination percentage, as well as in enlarging of the temperature range favorable to germination, is therefore less pronounced. The seeds of *B. lutea* seemed to be of low vitality from the start, so that differences in behavior under various conditions were less noticeable for this species.

Tables VII-X show the effect that various moist storage condi-

tions have upon the germination of *Betula* seeds at temperatures ranging from 15° to 32° C. Of the four different storage temperatures employed, 0° and 5° C. proved to be very much superior to 10° C., and to a constant frozen condition. This is shown by the high percentage of germination obtained at low germination temperatures

TABLE VII

GERMINATION OF *BETULA LENTA* SEEDS, COLLECTION OCTOBER, 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

TEMPERATURE DURING PERIOD OF MOIST STORAGE	WEEKS IN MOIST STORAGE	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alter- nation of 15°- 32° C.
Control (no moist storage) . . . . .		10	0	2	33	67
0° C. . . . .	4	80	80	77	72	75
	6	85	72	82	75	81
	8	77	79	81	82	77
	10	82	79	77	76	60
5° C. . . . .	4	83	75	77	72	77
	6	70	81	57	72	77
	8	72	77	74	54	56
	10	85	76	87	82	82
10° C. . . . .	4	7	22	24	58	78
	6	2	4	25	30	74
	8	10	10	16	54	72
	10	2	4	26	60	71
In frozen condition at -15 to -8°C. (soaked in water for a few hours at room temperature previous to storage) . . . . .	4	0	0	0	15	37
	6	0	0	1	28	69
	8	0	0	9	22	72
	10	0	2	31	28	55

after a period of moist storage at these temperatures. Seeds stored at 10° C. showed only a slight improvement of germination when transferred to the different germination chambers. For this reason 10° C. cannot be termed a good temperature for after-ripening of the species studied here. This observation differs from that of WEISS (13), who found that for the after-ripening of *B. populifolia* a moist storage period at 10° is as effective as one at 5° or at 0° C. Seeds stored below the freezing point failed completely to after-ripen.



When the samples were put into the cold chamber immediately after they had been placed on moist filter paper, so that no absorption of water could take place before the seeds were frozen, their quality was not altered at all when they were transferred into the germination chambers. When the seeds were allowed to absorb some mois-

TABLE VIII

GERMINATION OF *BETULA LENTA* SEEDS, COLLECTION NOVEMBER, 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

TEMPERATURE DURING PERIOD OF MOIST STORAGE	WEEKS IN MOIST STORAGE	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alter- nation of 15°- 32° C.
Control (no moist storage).....		1	0	4	4	12
0° C.....	4	45	22	13	6	17
	6	36	41	28	10	15
	8	Experiment lost				
	10	Experiment lost				
5° C.....	4	48	52	42	32	23
	6	42	45	36	29	37
	8	49	50	48	37	35
	10	80	50	48	32	38
10° C.....	4	37	22	44	18	45
	6	22	32	41	28	52
	8	46	30	41	28	38
	10	20	34	34	33	45
In frozen condition at -15° to -8° C. (soaked in water for a few hours at room temperature previous to storage).....	4	5	8	6	3	38
	6	7	3	6	3	19
	8	5	1	4	4	25
	10	8	8	4	1	55

ture, as in those samples recorded in tables VII-X, some injury could be noted in several samples. The amount of injury increased with the length of time the seeds were soaked at room temperature previous to freezing storage. An alternation of freezing and thawing killed samples of all three species. These results are not in agreement with those obtained by KINZEL (9) for various other seeds. He noted a beneficial effect of freezing and of an alternation between freezing and thawing.

The length of the cool moist storage period does not seem to be

of great importance. Four weeks proved to be long enough to after-ripen seeds at a favorable storage temperature, and ten weeks did not seem to be too long for a favorable result. In all later experiments six weeks of storage at 0° C. was selected as a suitable period for after-ripening.

TABLE IX

GERMINATION OF *BETULA Papyrifera* SEEDS, COMMERCIAL COLLECTION 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

TEMPERATURE DURING PERIOD OF MOIST STORAGE	WEEKS IN MOIST STORAGE	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alter- nation of 15°- 32° C.
Control (no moist storage).....		0	9	12	15.7	16.7
0° C.....	4	20	15	21	13	19
	6	24	22	25	19	41
	8	19.5	20	23	15	22
	10	29	20	23	21	19
5° C.....	4	38	36	33	26	36
	6	36	35	35	30	34
	8	49	44	41	44	45
	10	53	50	49	39	50
10° C.....	4	13	21	26	40	41
	6	19	25	20	33	52
	8	4	8	1	28	45
	10	Not started, material partly infected				
In frozen condition at -15° to -8° C. (soaked in water for a few hours at room temperature previous to storage).....	4	0	8	4	7	7
	6	0	1	6	7	7
	8	0	7	4	8	10
	10	0	6	3	7	8

The superiority of the October collection of *B. lenta* seeds over the other collections of the same species, which had been noted in previous tests, became again noticeable when samples of the different collections were after-ripened and then germinated, as is shown in table XI. The conditions under which the October collection was harvested differed from those of the other collections in the following manner: the catkins containing the seeds were dry and partly opened, and a small quantity of the seeds had been shed, while the seeds of the September collection were taken from fresh catkins

which were still closed, and those of the November collection from dried catkins, from which the greater part of the seeds had been shed. The seeds of the November collection had also been exposed to several nights of severe frost, while those of the October collection were harvested before frost had set in. According to this, the optimum

TABLE X

GERMINATION OF *BETULA LUTEA* SEEDS, COMMERCIAL COLLECTION 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

TEMPERATURE DURING PERIOD OF MOIST STORAGE	WEEKS IN MOIST STORAGE	PERCENTAGE GERMINATION AT				
		15° C.	20° C.	25° C.	32° C.	Alter- nation of 15°- 32° C.
Control (no moist storage).....		2	0	5	5	12
0° C.....	4	26	27	20	17	13
	6	22	21	17	14	14
	8	20	22	18	11	17
	10	14	19	2	7	0
5° C.....	4	5	10	11	12	17
	6	12	7	9	14	12
	8	11	11	10	12	10
	10	10	11	7	9	14
10° C.....	4	5	5	7	8	16
	6	4	5	7	8	17
	8	4.5	4.5	6	9	15
	10	4.5	8	9	11	12
In frozen condition at -15° to -8° C. (soaked in water for a few hours at room temperature previous to storage).....	4	0	1	5	5	3
	6	2	1	4	6	7
	8	1	1	3	4	7
	10	1	7	1	4	4

time for harvesting *B. lenta* seeds seems to be after the catkins have dried on the tree, but before they have opened far enough for a considerable part of the seeds to be shed.

All these experiments were conducted in the fall of 1926 and the spring of 1927, that is, during the first six months after harvest.

## 2. EXPERIMENTS WITH STORED SEEDS

In the fall of 1927 a new series of experiments was started for the purpose of determining the keeping quality of seeds from the same collections under various conditions. For these studies seeds

had been stored in open glass bottles at room temperature. They had also been kept, as previously described, with various amounts of hygroscopic moisture at room temperature, and in an ice chest with an average temperature of 8° C.

TABLE XI

GERMINATION OF DIFFERENT COLLECTIONS OF *BETULA LENTA* SEEDS AFTER-RIPENED FOR SIX WEEKS AT 0° C.; EACH PERCENTAGE IS AVERAGE OF TWO TESTS OF 200 SEEDS EACH

COLLECTION	PERCENTAGE GERMINATION AT				
	15° C.	20° C.	25° C.	32° C.	Alter- nation of 15°-32° C.
Collection A, September, 1926.....	54	62	57	50	67
Collection October, 1926.....	85	72	82	75	81
Collection November, 1926.....	58	44	45	24	30

TABLE XII

GERMINATION OF *BETULA* SEEDS, STORED DRY AT LABORATORY TEMPERATURE FOR ONE AND ONE-HALF YEARS; SEEDS NOT AFTER-RIPENED; GERMINATION TESTS MADE IN SPRING OF 1928; EACH PERCENTAGE IS AVERAGE OF TWO TESTS OF 200 SEEDS EACH

COLLECTION	PERCENTAGE GERMINATION AT				
	15° C.	20° C.	25° C.	32° C.	Alter- nation of 15°-32° C.
B. lenta, collection A, September, 1926, from green catkins.....	0	0	0	15	50
B. lenta, collection B, September, 1926, from brown catkins.....	0	0	0	7	25
B. lenta, collection October, 1926.....	0	0	4	46	76
B. lenta, collection November, 1926.....	0	0	0	3	16
B. papyrifera, commercial collection 1926..	0	11	15	16	19
B. lutea, commercial collection 1926.....	0	3	1	6	6

The results of the first sets of experiments are given in tables XII and XIII, showing the germination quality of the seeds which had been stored air-dry at room temperature for one and one-half years. They had not been protected against changes in temperature and atmospheric humidity during the entire storage period. Stored seeds, just like newly harvested laboratory-dry seeds, germinated well at 32° C., or an alternation of 15°-32° C., but showed an im-

provement in germination percentage and a great fall in the minimum germination temperature after a period of after-ripening. In figs. 1-3 the percentage of germination of new and stored seeds of various species and collections is compared. It is interesting to note that of the three collections of *B. lenta* made in 1926, the first and second kept their vitality perfectly, while the last collection lost it somewhat. This collection had the poorest quality from the beginning. The reason for this low vitality and poor keeping quality may be due to the period of rainy weather and several nights of frost to

TABLE XIII

GERMINATION OF BETULA SEEDS, STORED DRY AT LABORATORY TEMPERATURE FOR ONE AND ONE-HALF YEARS, THEN AFTER-RIPENED FOR SIX WEEKS AT 6° C.; GERMINATION TESTS MADE IN SPRING OF 1928; EACH PERCENTAGE IS AVERAGE OF TWO TESTS OF 200 SEEDS EACH

COLLECTION	PERCENTAGE GERMINATION AT				
	15° C.	20° C.	25° C.	32° C.	Alter- nation of 15°-32° C.
<i>B. lenta</i> , collection A, September, 1926, from green catkins. . . . .	52	52	54	41	54
<i>B. lenta</i> , collection B, September, 1926, from brown catkins. . . . .	20*	18	19	15	17
<i>B. lenta</i> , collection October, 1926. . . . .	81	83	86	78	77
<i>B. lenta</i> , collection November, 1926. . . . .	44	34	33	16	25
<i>B. papyrifera</i> , commercial collection 1926. . . . .	23	19	17	10	15
<i>B. lutea</i> , commercial collection 1926. . . . .	3	5	4	3	4

\* Some fungal infection in this collection.

which these seeds had been exposed on the tree; or to the fact that the heavier and better developed seeds had dropped out of the catkins before this last collection was made.

Of all the species studied, *Betula papyrifera* lost its vitality quickest. In one year of storage the germination dropped from 81 to 19 per cent at the optimum germination temperature. This is interesting in view of the fact that *B. papyrifera* has at the same time the least dormant seeds of the four species. The quality of *B. populifolia* seeds has improved in germination rather than decreased, while that of *B. lutea* is slightly lower.

It has been stated that all freshly harvested seeds improved equally during the first month of dry storage in the laboratory, re-

ardless of the amount of hygroscopic moisture present in the seed at the time of harvest. When the storage period was lengthened,

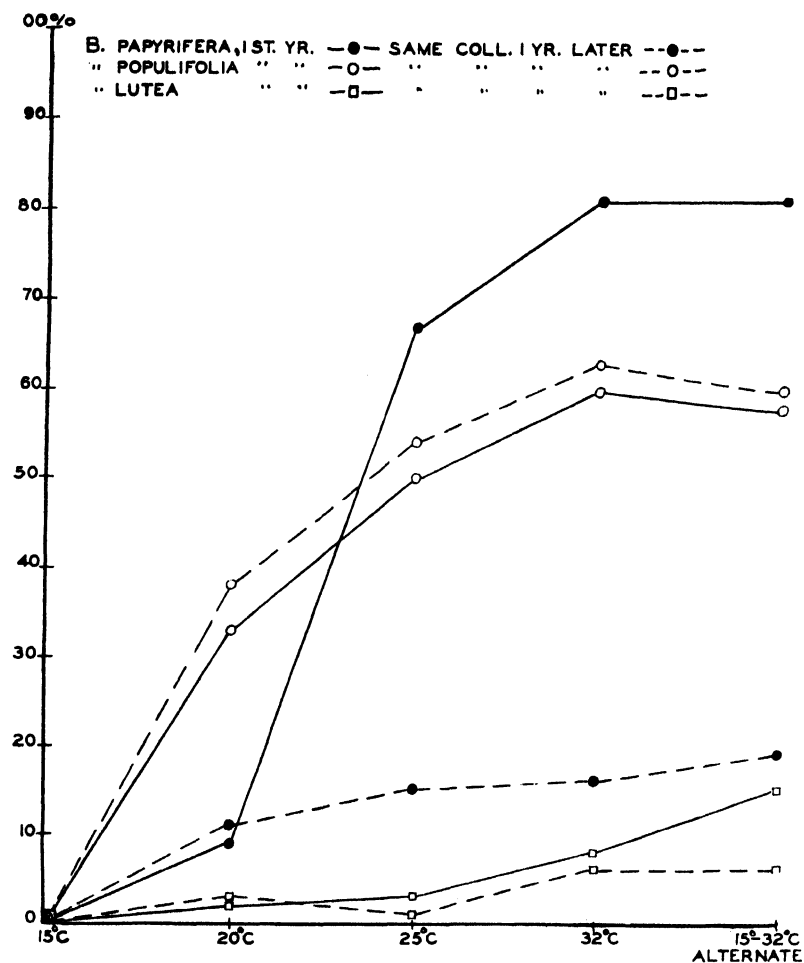


FIG. 1.—Germination of unafter-ripened *Betula* seeds at various temperatures shortly after harvest and after one year of storage at room temperature.

however, the amount of hygroscopic moisture contained in the various samples had a pronounced influence upon the changes going on in the seed during the storage period. Table XIV gives the water content expressed in percentage of dry weight of three species of

*Betula* as it was obtained by storage over CaO and different solutions of  $H_2SO_4$ .

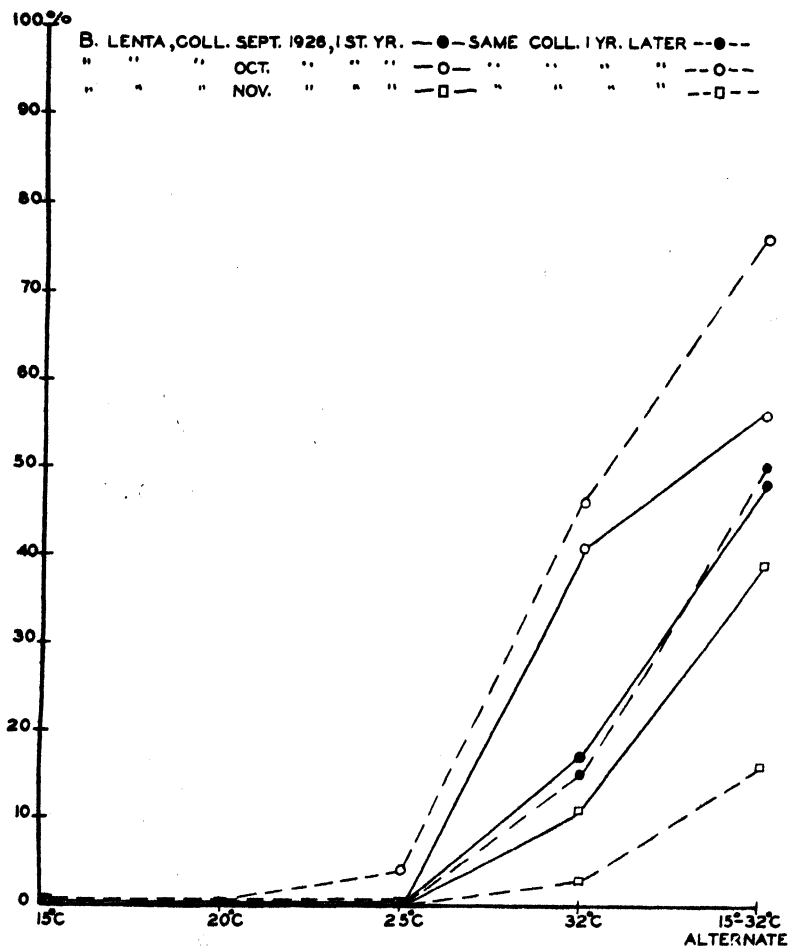


FIG. 2.—Germination of different collections of unafter-ripened *Betula lenta* seeds at various temperatures shortly after harvest and after one year of storage at room temperature.

The keeping quality of each sample from this series was determined for two different storage temperatures, room temperature and ice box temperature, and the results obtained are tabulated in tables

XV, XVI, and XVII. There was also one series of samples stored in a frozen condition at a temperature varying from  $-15^{\circ}$  to  $-8^{\circ}$  C.,

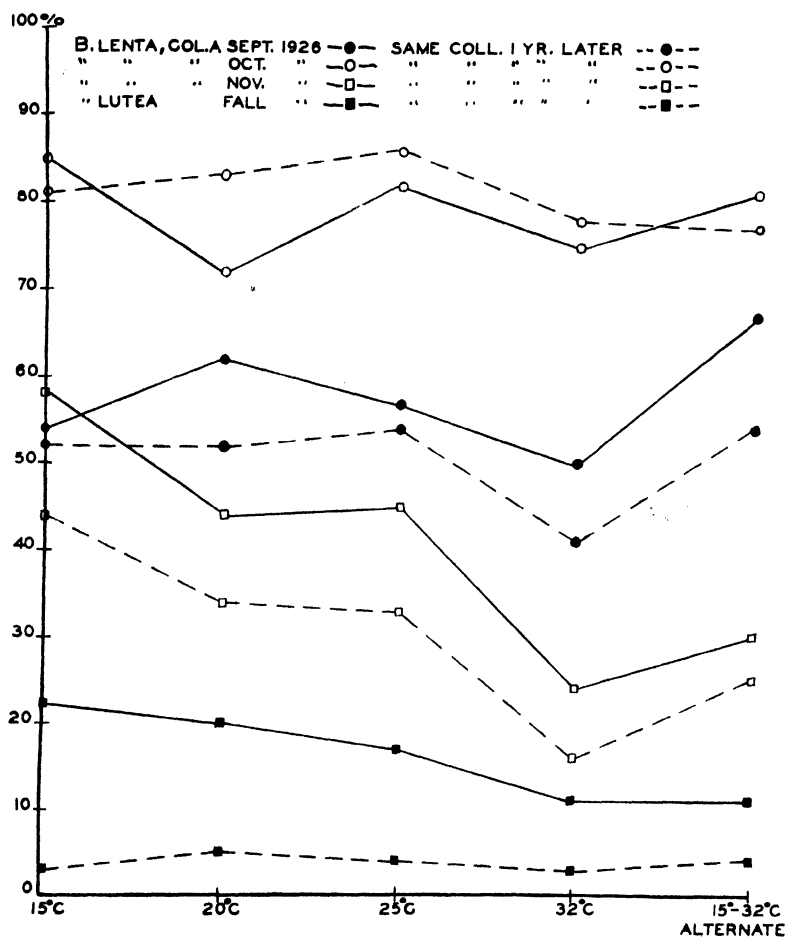


FIG. 3.—Germination of *Betula lutea* and different collections of *B. lenta* seeds at various temperatures shortly after harvest and one year after harvest; all samples after-ripened for six weeks at  $6^{\circ}$  C. before being transferred to germination chamber.

but since germination tests showed that most of the seeds stored at ice box temperature had not lost in vitality, this last set of samples was left unopened to be tested after a storage period of two years or more. The data in table XV show clearly that seeds of *Betula lenta*,



stored with a moisture content of 8.2 per cent or lower, keep perfectly well during the first year of storage at room temperature. With

TABLE XIV

AMOUNT OF HYGROSCOPIC MOISTURE IN BETULA SEEDS IN EQUILIBRIUM WITH VARIOUS CONCENTRATIONS OF  $\text{H}_2\text{SO}_4$  AND WITH QUICKLIME

DRYING AGENT	RELATIVE HUMIDITY OVER SOLUTION AT ROOM TEMPERATURE	HYGROSCOPIC MOISTURE IN PERCENTAGE DRY WEIGHT		
		<i>B. lenta</i> , October	<i>B. papyrifera</i>	<i>B. populifolia</i>
$\text{CaO}$ .....	0	0.01	0.6	0.4
Concentrated $\text{H}_2\text{SO}_4$ ..	0	0.6	0.7	0.1
64.8% $\text{H}_2\text{SO}_4$ .....	10	4.4	4.8	5.2
55.9% $\text{H}_2\text{SO}_4$ .....	25	6.3	6.4	7.2
		(original moisture content 7.09)		(original moisture content 7.36)
43.4% $\text{H}_2\text{SO}_4$ .....	50	8.2	8.7	8.5
			(original moisture content 8.7)	
30.4% $\text{H}_2\text{SO}_4$ .....	75	11.8	11.9	11.5
18.5% $\text{H}_2\text{SO}_4$ .....	90	17.6	17.8	17.8

TABLE XV

GERMINATION OF BETULA LENTA SEEDS, COLLECTION OCTOBER, 1926, SEEDS OF VARIOUS MOISTURE CONTENTS STORED AIR-TIGHT FOR ONE AND ONE-HALF YEARS AT ROOM TEMPERATURE AND IN ICE BOX; SEEDS NOT AFTER-RIPENED; 400 SEEDS IN EACH TEST

DRYING AGENT	MOISTURE CONTENT	PERCENTAGE GERMINATION AFTER STORAGE AT ROOM TEMPERATURE				PERCENTAGE GERMINATION AFTER STORAGE IN ICE CHEST			
		15° C.	20° C.	32° C.	Alteration of 15°-32° C.	15° C.	20° C.	32° C.	Alteration of 15°-32° C.
$\text{CaO}$ .....	0.01	0	2	62.5	80.5	0	4.5	38	74.5
Concentrated $\text{H}_2\text{SO}_4$ ..	0.6	0	4	62.5	73	0	6.5	47.5	74.5
64.8% $\text{H}_2\text{SO}_4$ .....	4.4	0	2	53.5	84	0	2	50	79
55.9% $\text{H}_2\text{SO}_4$ .....	6.3†	0	0	57	68.5	0	2	69	84
43.4% $\text{H}_2\text{SO}_4$ .....	8.2	0	1	32	76	0	0	43	74
30.4% $\text{H}_2\text{SO}_4$ *.....	11.8	0	0	0	0	0	0	70	77
18.5% $\text{H}_2\text{SO}_4$ *.....	17.6	0	0	0	0	0	0	2	5

\* Seeds kept for entire storage period over solutions with higher vapor pressure than that of laboratory air.

† Original moisture content of *B. lenta*, collected October, 1926, 7.09.

a moisture content of 11.8 per cent seeds do not keep in room temperature, but retain part of their viability at 8° C. With 17.6 per cent

of moisture seeds lose their vitality in low temperature storage as well as at room temperature.

TABLE XVI

GERMINATION OF *BETULA POPULIFOLIA* SEEDS, COLLECTION OCTOBER, 1926, OF VARIOUS MOISTURE CONTENTS STORED AIR-TIGHT FOR ONE AND ONE-HALF YEARS AT ROOM TEMPERATURE AND IN ICE CHEST; SEEDS NOT AFTER-RIPENED; 400 SEEDS IN EACH TEST

DRYING AGENT	MOISTURE CONTENT	PERCENTAGE GERMINATION AFTER STORAGE AT ROOM TEMPERATURE				PERCENTAGE GERMINATION AFTER STORAGE IN ICE CHEST			
		15° C.	20° C.	32° C.	Alter-nation of 15°-32° C.	15° C.	20° C.	32° C.	Alter-nation of 15°-32° C.
CaO.....	0.4	0	26.5	48.5	43	0	17.5	66.5	77.5
Concentrated H <sub>2</sub> SO <sub>4</sub> ..	0.1	0	23	46	36	0	8.5	49.5	44
64.8% H <sub>2</sub> SO <sub>4</sub> .....	5.2	0	23	73	68	0	14.5	57.5	63.5
55.9% H <sub>2</sub> SO <sub>4</sub> .....	7.2†	0	38	63	47	0	37.5	82	64.5
43.4% H <sub>2</sub> SO <sub>4</sub> .....	8.5	0	12	44	43	0	13	47.5	71.5
30.4% H <sub>2</sub> SO <sub>4</sub> *.....	11.5	0	0	0	0	0	22.5	53.5	71.5
18.5% H <sub>2</sub> SO <sub>4</sub> *.....	17.8	0	0	0	0	0	2	7.5	9.5

\* Seeds kept for entire storage period over solutions with higher vapor pressure than that of laboratory air.

† Original moisture content of *B. populifolia*, 1926, 7.2.

TABLE XVII

GERMINATION OF *BETULA PAPYRIFERA* SEEDS OF VARIOUS MOISTURE CONTENTS STORED AIR-TIGHT FOR ONE AND ONE-HALF YEARS AT ROOM TEMPERATURE AND IN ICE CHEST; SEEDS NOT AFTER-RIPENED; 400 SEEDS IN EACH TEST

DRYING AGENT	MOISTURE CONTENT	PERCENTAGE GERMINATION AFTER STORAGE AT ROOM TEMPERATURE				PERCENTAGE GERMINATION AFTER STORAGE IN ICE CHEST			
		15° C.	20° C.	32° C.	Alter-nation of 15°-32° C.	15° C.	20° C.	32° C.	Alter-nation of 15°-32° C.
CaO.....	0.6	0	51	64.5	69	1	35.5	70	77.5
Concentrated H <sub>2</sub> SO <sub>4</sub> ..	0.7	0	33	63.5	79	0	63	92.5	83.5
64.8% H <sub>2</sub> SO <sub>4</sub> .....	4.8	0	12	32	49	0	37	77	77.5
55.9% H <sub>2</sub> SO <sub>4</sub> .....	6.4	0	5	8	29	0	78.5	84	91
43.4% H <sub>2</sub> SO <sub>4</sub> .....	8.7†	0	3	7.5	11	0	41	81.5	64
30.4% H <sub>2</sub> SO <sub>4</sub> *.....	11.9	0	0	0	0	0	26	82.5	80
18.5% H <sub>2</sub> SO <sub>4</sub> *.....	17.8	0	0	0	0	0	4.5	6	6.5

\* Seeds kept for entire storage period over solutions with higher vapor pressure than that of laboratory air.

† Original moisture content of *B. papyrifera*, commercial collection 1926, 8.7.

The behavior of *B. populifolia* is somewhat different from that of *B. lenta*, as is shown in table XVI. The keeping quality of *B. populi-*

*folia* was reduced by storage with a very low hygroscopic moisture, as well as by storage with a water content as high as that of freshly harvested seeds and higher. The optimum moisture content for storage at room temperature seems to be about 5.2 per cent. With seeds kept in the ice box, the importance of the water content decreases for *B. populifolia* in the same way that it does for *B. lenta*.

A third type of reaction is obtained when seeds of *B. papyrifera* are stored with different amounts of hygroscopic moisture (table XVII). Seeds stored at room temperature keep well with greatly reduced moisture content only, lose a considerable amount of vitality when stored with the original moisture content of freshly harvested seeds, and die completely when stored with increased water content.

The three types of keeping quality may be characterized briefly in the following way:

TYPE 1 (*B. lenta*).—At ordinary room temperature seeds keep well with a moisture content ranging from 0.01 to 8.2 per cent (m.c. at time of harvest 7.9 per cent). With a m.c. higher than 8.2 per cent seeds keep only when stored at ice chest temperature.

TYPE 2 (*B. populifolia*).—At ordinary room temperature seeds keep well only at the slightly reduced moisture content of about 5.2 per cent (m.c. at time of harvest 7.2 per cent). With a m.c. reduced below or increased above 5.2 per cent seeds keep well only at ice chest temperature.

TYPE 3 (*B. papyrifera*).—At ordinary room temperature seeds keep well only with a highly reduced moisture content of 0.6 or 0.7 per cent (m.c. at time of harvest 8.7 per cent). With a m.c. above 0.7 per cent seeds keep well only at ice chest temperature.

At a moisture content of about 17.6 per cent all three types lose their vitality completely at room temperature, and almost completely at ice chest temperature, within the first year of storage. A comparison of germination obtained at the optimum germination temperature of 15°–32° C. alternation in all three species of *Betula* stored at various moisture contents in room temperature is given in the curves shown in fig. 4. Fig. 5 shows the modifying influence of a low storage temperature under otherwise similar conditions.

It is of interest to note that although seeds containing a high amount of hygroscopic moisture keep comparatively well at a cool

storage temperature, they do not after-ripen, as is shown by the fact that no germination is obtained at  $15^{\circ}\text{C}$ . as is characteristic for

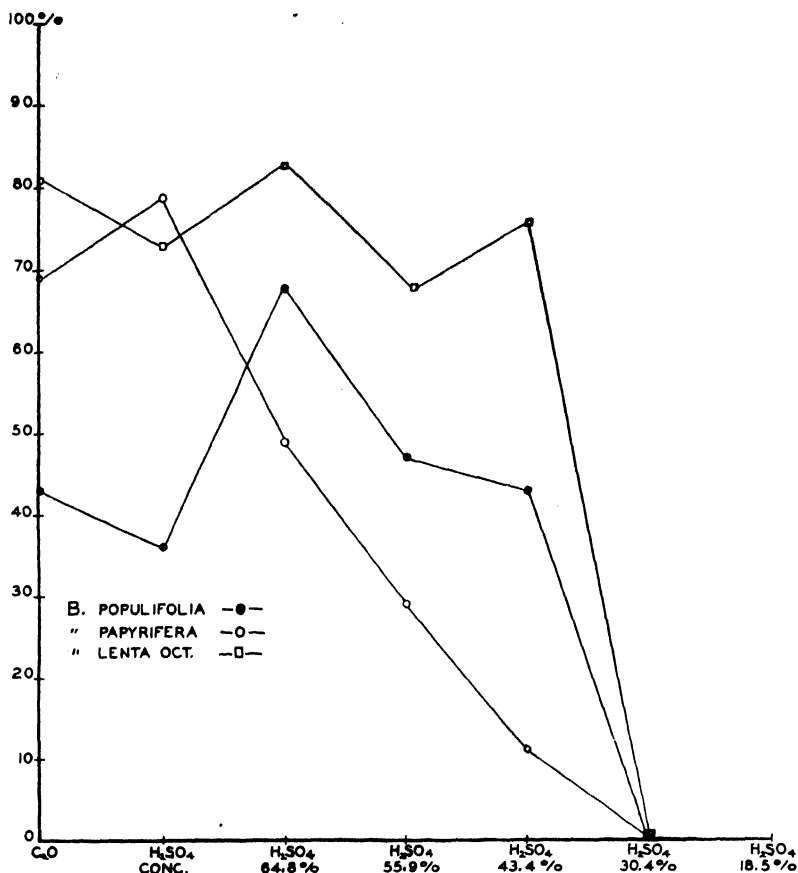


FIG. 4.—Germination of *Betula* seeds after one year of storage at room temperature in hermetically sealed vessels in atmospheres of different humidities; germination at alternating temperatures,  $15^{\circ}$ – $32^{\circ}\text{C}$ . (eighteen hours a day at  $15^{\circ}$  and six hours a day at  $32^{\circ}\text{C}$ .).

after-ripened seeds. To secure after-ripening at low temperatures a much higher moisture content of the seeds is required.

### Discussion

In a discussion of the results obtained in these experiments, three facts call for special attention: (1) the behavior of freshly harvested

seeds as compared with laboratory-dry seeds; (2) the effect of moist cool storage upon the temperature requirements of germination; and

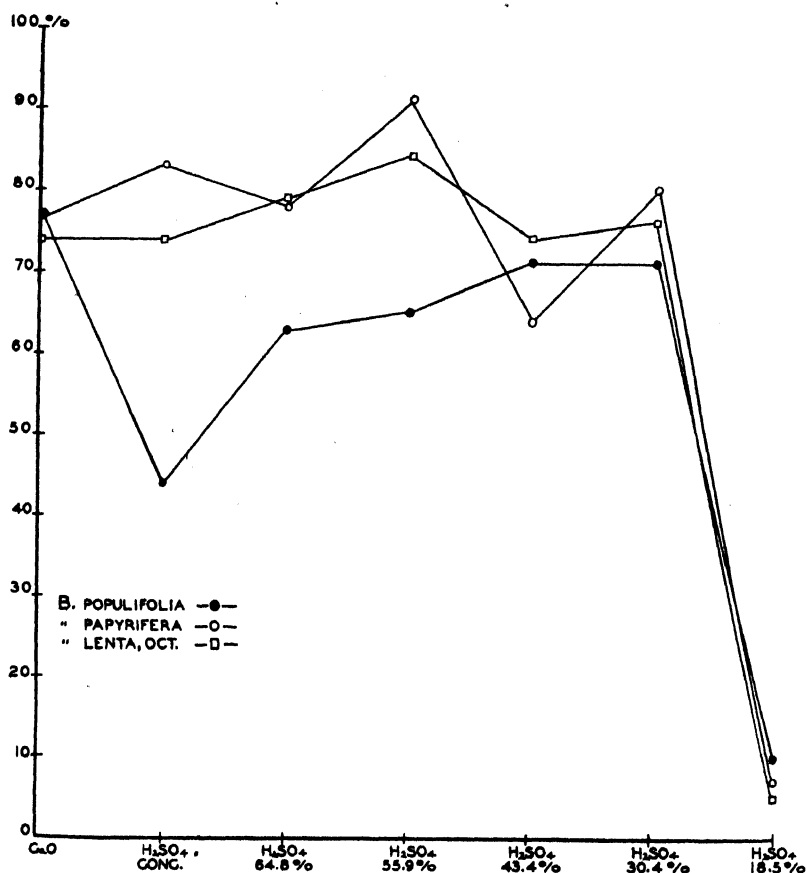


FIG. 5.—Germination of *Betula* seeds after one year of storage in ice chest in hermetically sealed vessels in atmospheres of different humidities; germination at alternating temperatures, 15°–32° C. (eighteen hours a day at 15° and six hours a day at 32° C.).

(3) the relation of moisture content to keeping quality in the different species studied.

It has been shown that fresh seeds of *B. lenta* germinate very poorly immediately after harvest, but improve considerably in germination quality during one month of dry storage. Causes for this improvement may be due to internal changes as well as to an

alteration in seed coat characters. If the changes are internal, they must be different from those that could be produced through variations in moisture content in the seed, or through the influence of frost and other weather conditions while the seeds are still attached to the tree, since different samples of seeds harvested at different times of the season and containing various amounts of hygroscopic moisture were all improved by one month of dry storage. Changes in the seed coat, such as an increase in permeability to water, oxygen, or carbon dioxide produced by a continuous drying at temperatures higher than those prevailing out-of-doors during the harvesting season, may also be responsible for the beneficial effect of dry storage. Unfortunately the small size of birch seeds and the tightness with which the coat fits around the embryo made it impossible to remove the coat of fresh seeds successfully in order to find out whether or not the fresh coat was responsible for the failure of the seeds to germinate.

Many seeds require low temperature stratification to prepare them for germination. This is true of rosaceous seeds (1, 2, 5, 6, and 7), *Tilia* (11), *Juniperus* (10), *Acer* (8), *Cornus*, *Sambucus*, and *Berberis* (3, 4). In all of these high temperatures are ineffective in forcing germination previous to after-ripening in low temperature stratification. *Betula* species differ from this type in so far as they need a period of cool storage only to enable the seed to germinate at the lower temperatures, as 15° and 20° C. At temperatures such as 32° C. and an alternation of 15°–32° C. the seeds germinate without previous treatment. When the samples are kept moist at after-ripening temperatures, such as 0°, 5°, or 10° C. for longer periods than those recorded in this paper, the seeds finally begin to germinate at the storage temperatures, those stored at 10° growing first, and those stored at 0° C. beginning to grow only after 5–6 months.

In this study it was found that the species which required cool storage for later germination at the lower temperatures proved most resistant when stored under various conditions, while the less "dormant" species, *B. papyrifera*, lost its vitality much easier under unfavorable conditions of storage. A physiological or chemical basis for the relation of keeping quality to dormancy has not been worked out.

## Conclusion

### I. GERMINATION

1. Freshly harvested seeds of *Betula lenta* germinate poorly regardless of time of harvest, moisture content, and dry weight of the seeds at harvest time. The germination quality improves considerably during the first month of dry storage at laboratory temperature.

2. The optimum germination temperature for air-dried seeds of *B. lenta*, *B. papyrifera*, *B. lutea*, and *B. populifolia* is 32° C. constant, or alternation between 15° and 32° C.

3. The minimum germination temperature for dry-stored seeds is remarkably high, being about 30° for *B. lenta*, and about 20° C. for *B. populifolia*, *B. papyrifera*, and *B. lutea*. The dry-stored seeds have a narrow temperature range for germination.

4. When air-dry or freshly harvested seeds of *Betula* are kept in an imbibed condition at low temperatures they after-ripen, or go through a series of changes that improves their germination at high temperatures and enables them to germinate at much lower temperatures. The most favorable temperatures for after-ripening are 0° to 5° C., while 10° is less favorable, and storage in a frozen condition is ineffective or even injurious. Four weeks of stratification at 0° or 5° puts the seeds into condition for excellent germination at 15° C. Six to eight weeks of stratification at these temperatures reduces the minimum germination temperature sufficiently to give good early spring germination in outdoor seed beds. Seeds stratified at 0° C. for 5-6 months begin to germinate profusely even at this low temperature. In short, the minimum germination temperature falls 20°-30° C. with such treatment.

5. Moist sand, granulated peat, or blotting or filter paper are equally effective as stratification media, so that the seeds are indifferent to a considerable range of acidity in the medium.

6. Seeds of *B. papyrifera* are less dormant than are those of the other species studied. They also fall in vitality most rapidly in unfavorable storage condition.

7. The falling of the germination minimum with low temperature stratification must be advantageous to the seeds in nature. The high germination minimum of the freshly shed or dry seeds will insure no germination in the fall. The cold weather of the winter, with the

seeds buried under leaves and snow, will after-ripen them and prepare them for early spring germination. This probably accounts for the abundance of *Betula* seedlings in the early spring. Knowledge of the after-ripening at low temperature stratification is also very important to the producer of birches. Dry-stored seeds sown outside in early spring will not germinate because of the high temperature minimum, while properly stratified seeds will give quick and abundant germination in early spring.

8. The percentage germination of *Betula* seeds reported in this paper is very much higher than that reported in forestry books (12). This is probably easily explained by the facts that past workers have used too low temperatures for the germination of dry-stored seeds, or they have failed properly to after-ripen the seeds that are to be germinated at low temperatures in the seed beds in the early spring.

9. Germination of dry-stored *Betula* seeds is not affected by a considerable range of acidity of the medium in which germination takes place; neither is it influenced by light, increased CO<sub>2</sub>, or a considerable range of O<sub>2</sub> pressure. Sterilization with 0.2 per cent uspulun for one-half hour causes a slight reduction in percentage of germination.

## II. STORAGE

10. During one year of air-dry storage at room temperature, seeds of *B. lenta* and *B. populifolia* kept perfectly, while *B. lutea* and *B. papyrifera* fell in viability.

11. The optimum moisture content for seeds stored at room temperature in sealed containers lies considerably below the moisture content of freshly harvested seeds for *B. papyrifera* (0.6 per cent), while *B. populifolia* keeps best with a medium amount of hygroscopic moisture (5.2 per cent). *B. lenta* keeps well in all except very humid conditions during a storage period of one year.

12. Stored at ice box temperature, seeds with higher moisture content keep as well as seeds low in hygroscopic moisture for one and one-half years of storage.

## III. SUGGESTIONS FOR GROWERS

13. Birch seeds are harvested to best advantage after the catkins have begun to dry on the trees, but before they have shed a con-



siderable part of the seeds. They should be shaken from the catkins, dried on well ventilated racks, and stored. About six weeks before planting the seeds ought to be stratified in a suitable moist medium at temperatures of from 32° to 41° F. After such a treatment the seeds will germinate in the seed beds in early spring. It takes approximately one month to six weeks for a full stand of young seedlings to appear above the surface of the soil. Early spring planting is recommended.

14. For dry storage of seeds for a few months no special precautions need be taken, but for storage of one year or more the following methods ought to be used: *B. lenta* can be stored in almost any storage room of average room temperature that is dry and well ventilated; *B. populifolia* is sensitive to excessive drying as well as to very high humidities, and should therefore be kept in closed containers at a cool temperature; *B. papyrifera* keeps best when thoroughly dry, which can be done by keeping the seeds suspended in a bag in a closed container, the bottom of which is covered with quick lime.

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## AN INEXPENSIVE AND QUICKLY MADE INSTRUMENT FOR TESTING RELATIVE HUMIDITY<sup>1</sup>

WILLIAM B. SHIPPY

(WITH THREE FIGURES)

It is commonly understood that sulphuric acid and various salts may be used in the control of humidity. WILSON<sup>2</sup> discusses the use of sulphuric acid in humidity control, and SPENCER<sup>3</sup> presents a list of inorganic salts supplying a rather wide range of humidities.

Most investigators attempting to control humidity do not actually make humidity readings. The usual procedure is to check the specific gravities of the control solutions from time to time. It is taken for granted that a given concentration of solution will give a specified vapor pressure, but such an assumption may lead to error. To illustrate: A given concentration of sulphuric acid solution under specific temperature and pressure conditions will exert a definite humidity control through desiccation of a limited volume of air. Any modification of these prescribed conditions will necessarily result in a humidity variation of the air under control; hence, any decrease or increase in temperature brings about a corresponding change in vapor pressure. In addition to the preceding, there is often the possibility of leakage in tubing, ill-fitting stoppers, or defective glassware. If living plant material is placed within the controlled vessel, large quantities of water will be given off through transpiration. A further source of difficulty lies with inaccuracies due to the control solution itself, which may contain impurities causing it to give a higher specific gravity reading than would correspond to its sulphuric acid content. Defective recording instruments also might

<sup>1</sup> This work was done in connection with a study sponsored jointly by the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, and the Crop Protection Institute, and published at the expense of the former Institute out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> WILSON, R. E., Humidity control by means of sulfuric acid solutions, with critical compilation of vapor pressure data. Jour. Indus. and Engin. Chem. 13:326-331. 1921.

<sup>3</sup> SPENCER, H. M., Laboratory methods for maintaining constant humidity. Internatl. Critical Tables 1:67-68. 1926.

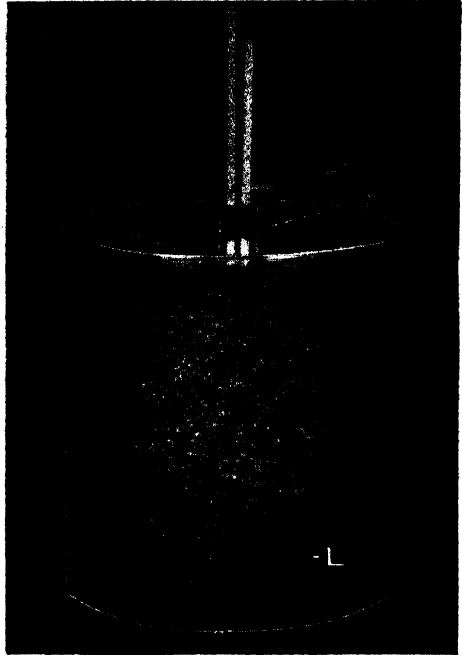
mislead one. In the last analysis it is not the potential desiccation power of the control solution that really matters; it is the humidity actually present within the controlled vessel, and this can only be determined by measurement. Thus it is apparent that difficulties lie in the pathway of any one attempting accurate humidity control. Specific gravity determinations of a control solution are not sufficient evidence under ordinary experimental conditions that a definite vapor pressure has been attained.

Various instruments are available for humidity testing, including the dew-point apparatus, instruments of the wet and dry bulb principle, and various types of hair hygrometers. These vary in reliability, but among the best are those of the wet and dry bulb type.

The writer began studies requiring constant humidities during the spring of 1927. It was found desirable to use an arrangement providing for a continuous flow of air through the controlled vessels, and a need arose for some instrument which would check the humidities delivered by the control solutions. The instrument here described, employing the principle of the wet and dry bulb, was designed to fill this need. It is illustrated in figs. 1-3.

An ordinary  $8\frac{1}{4}$ -inch pyrex test tube (fig. 1D) was drawn out at the bottom and joined with a  $1\frac{1}{4}$ -inch neck of quarter-inch glass tubing (H). The test tube was fitted with a three-hole rubber stopper (C). Two  $100^{\circ}$  F. matched thermometers (A) and an elbow of quarter-inch glass tubing (B) were inserted through the stopper. The thermometers were so arranged that when the tube was stoppered, the dry bulb would be approximately three inches and the wet bulb (carefully wrapped with one thickness of clean muslin, which had been thoroughly soaked in water) four inches from the base of the tube. For convenience in holding the instrument, it was commonly placed within an empty suction flask (fig. 3N).

Air forced by compression (applied at M) or drawn by vacuum (applied at O) from the control solutions (P) through this test tube *past the bulbs from the bottom inlet (H) to the top outlet (B)* gives a reading within a few minutes, the time depending on the rate of air flow. The rate of air flow does not call for great precision, but uniformity of flow is desirable. A flow of one liter per minute (moderately rapid bubbling) is a good rate to use in



FIGS. 1-3.—Fig. 1, instrument for testing humidity: *A*, two matched thermometers; *B*, air outlet tube to which vacuum tube is attached; *C*, 3-hole rubber stopper; *D*, pyrex test tube; *E*, wet bulb; *F*, dry bulb; *G*, fine-mesh copper screen plug; *H*, air inlet tube. Fig. 2, testing relative humidity within solid medium: *J*, point at which vacuum tube is attached; *K*, approximate depth instrument is buried in medium; *L*, peat moss medium. Fig. 3, testing relative humidity of air passing through control solution: *M*, air inlet where compressed air tube may be attached; *N*, empty suction flask used to hold instrument; *O*, air outlet where vacuum tube may be attached *P*, control solution.

checking most solutions, but satisfactory readings may be made over a fairly wide range. In testing a solution of low vapor pressure, it is not desirable to use too slow an air flow, as the cloth on the wet

TABLE I

TYPICAL SET OF READINGS WITH INSTRUMENT COMPARED WITH THEORETICAL RELATIVE HUMIDITIES AS INDICATED BY SPECIFIC GRAVITIES OF CONTROL SOLUTIONS

OCTOBER 18, 1928; RATE OF AIR FLOW ONE LITER PER MINUTE

DETERMINATIONS BY INSTRUMENT					DETERMINATIONS BY SPECIFIC GRAVITY	
Readings		Depression of wet bulb	Room temperature	Relative humidity* (per cent)	Specific gravity of control solution	Corresponding relative humidity
Wet bulb	Dry bulb					
Solution I (water)						
74.5* 76.75†	75.5* 77 †	0.25	77	99	1.000	100
75.5 77.25	77 77.5	0.25	77	99	1.000	100
Solution II (sulphuric acid)						
78 73.5	80 79	5.5	79	78	1.190	81
75 74	79 78.75	4.75	79	81	1.190	81
77 73.5	77.5 78.25	4.75	78	81	1.190	81
Solution III (sulphuric acid)						
79 71.5	80 80	8.5	80	68	1.265	65
75 71.5	80 80	8.5	80	68	1.265	65
75 70	79 78	8.0	78	69	1.265	65

\* Initial readings.

† Final readings.

<sup>†</sup> MARVIN, C. F., Psychrometric tables for obtaining the vapor pressure, relative humidity, and temperature of the dew-point. U.S. Weather Bureau, no. 235. 78-79. 1915.

WILSON, R. E., Graph on page 328 (article cited).

bulb may dry before time has been allowed for the reading. The reading is made when both mercury columns have come to rest. This makes possible a quick and satisfactory check on the humidity delivered by a control solution. To illustrate how the tester is used, a typical set of determinations on three solutions is shown in table I.

The instrument described can be used for testing relative humidity within solid media. If the investigator desires to know the humidity within a solid medium, as peat moss or a fairly open soil, the basal neck of the tube (H) may be fitted with a plug of fine-mesh copper screen (fig. 1G) to prevent solid particles from entering. The instrument is forced down into the medium (fig. 2L) almost to the top (K), the vacuum tube applied (at J) and a reading made. Such tests are useful in work on the stratification of seeds or in the storage of cuttings, root-grafts, or other similar materials. It may be said in this connection that there is danger of inaccuracy if small volumes of medium are tested, as the replacement of air between the medium particles from the outside would be too great.

One may test with this instrument the relative humidity of large or small volumes of air, 5-10 liters being sufficient for a reading. The size and shape of the instrument make possible its insertion through small openings, such as the hole in the lid of a desiccator or the top of a bell jar. Thus readings can readily be made out of doors or within constant condition chambers of large or small size. In addition to a rather wide use as a tester of humidity, it has the dependability of the sling psychrometer. It may be quickly constructed in any laboratory and is made of standard equipment in common use.

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## RÔLE OF MOTHER TUBER IN GROWTH OF POTATO PLANT<sup>1</sup>

F. E. DENNY

(WITH FIVE FIGURES)

### Introduction

Substances stored in the mother tuber are utilized by the sprout as soon as germination begins, and many experiments have shown that the sturdiness of the sprout, the subsequent rate of growth, and the final yield are related to the size of the seed-piece at the time of planting. Questions relating, however, to the stage of development at which the sprout becomes entirely independent of the mother tuber, the nature of the substances passing to the sprout which are so influential in modifying its growth, the rate at which different compounds pass from the tuber to the sprout, and the correlation in point of time which may exist between this transfer and the subsequent growth of the plant, these questions either find no answer in the literature of the subject or the answers that have been provided are conflicting.

By a series of experiments carried out in 1926, 1927, and 1928, it is believed that additional information upon these points has been obtained. The method of study has been, in brief, to amputate the mother tuber (by a method which involved a minimum disturbance of the root system) at different intervals after planting, that is, at different stages in the development of the plant, to observe the effects of this amputation upon the subsequent growth and final yield by comparison with check plants from which mother tubers had not been removed, to determine by chemical analyses of the amputated mother tissue what substances had disappeared and at what rate, and finally to make use of these observations to determine the periods at which connection with the mother tuber is critical,

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.



and to correlate the chemical composition at any period with behavior after amputation.

In these experiments an effort was made to deal with sufficiently large numbers of plants so that the influence of experimental errors would be reduced. During the three years, about 3000 plants, including amputated and checks, have been used in the tests. Consequently the samples of tissue taken for analyses have represented large numbers of individual tubers, and, since the yield data were obtained separately for each plant, the influence of variability (which is high in experiments on potato yield) could be measured and allowed for. Furthermore, although only two varieties, Irish Cobbler and Bliss Triumph, were used in the main experiments, preliminary tests were also made with ten other varieties, and it is likely that the results can be considered applicable to a considerable number of varieties. In one respect these experiments have been narrowly restricted, however, in that in nearly all cases only one size of seed-piece has been used, namely, 28 gm. The influence of size of seed-pieces upon these results requires further experimentation.

### Summary of literature

The attempt is not made here to deal chronologically with the various papers that have been published on this and related subjects, but rather to indicate the relation which they bear to the particular phases concerned in the present experiments, and to show the status of the subject at the time the work was initiated.

Amputation experiments were carried out by SIKORSKI (18), who on May 20 planted six potato tubers (weight 63-66 gm.), three in soil and three in sand. He removed the mother tuber from one plant in each series on June 30, and from another plant on August 31. As compared with plants from which mother tubers were not removed, it was found that amputation had reduced the yield in all cases. He believed that the influence of the mother tuber is not restricted to the early stage of development, but extends over the complete vegetative period. He suggested that in the later stages the mother tuber acts as a storage organ for water, and that this function is very important in periods of drought.

More recently SELIBER (16, 17) has furnished two reports of his

amputation experiments, in which larger numbers of plants were dealt with. His method of removing the mother tuber from the growing plant was to undermine the plant by removing earth until the mother tuber could be removed by hand. Two types of check plants were used, one being plants that were undermined in the same way until the mother tuber was touched by the hand but was not removed, and the other being plants which grew continuously in the soil without disturbance. He found that the removal of the mother tuber, even in midseason, reduced the final yield; but that the variety of potato was an important factor, the varieties Reitan and Chugunka being influenced to a great extent by amputating the mother tuber, while in the varieties Kruger and Epicurez the substance of the mother tuber was not utilized. With these latter varieties, presence or removal of the mother tuber was not a matter of importance, so far as the yield was concerned. SELIBER also discusses the importance of the mother tuber as a water storage organ.

The literature relating to the extent to which the substance of the mother tuber is utilized, that is, as to the amount of reduction in dry weight during the growing season, is conflicting. The earlier workers were impressed by the large proportion of dry substance that is removed from the mother tuber, while certain more recent reports tend to show that considerable amounts of the stored foods remain in the seed-piece even at the end of the growth period.

As an example of the first group, we may cite FITTBOGEN, GROENLAND, and FRAUDE (9), who carried out the first and most complete set of analyses of mother tubers at intervals during the growth of the plant. They planted the tubers on April 13 and dug up sample lots of plants on May 28, June 18, July 2, July 25, August 20, and September 22. The analyses of the mother tubers on these dates showed a rapid decrease in protein, starch, fat, "undetermined organic," and ash. More than 70 per cent of the organic substance of the mother tuber had been lost by June 18.<sup>2</sup>

MÜLLER (12) noted that even at the end of a dry summer the mother tubers that were recovered from potato vines at harvest were not shrunk, but were full of sap. But his analyses of the recovered

<sup>2</sup> Their analyses show that in spite of this loss of dry matter, the sugar content of the mother tubers increased, and the present experiments confirm this finding.

seed-pieces showed that the dry substance represented about 3.7 per cent of the fresh weight, that almost all of the starch, protein, and phosphorus compounds had disappeared, and that ash constituents (except KCl!) had been reduced to about one-half the original amount.<sup>3</sup>

In one of the more recent articles relating to the utilization of the stored food, JONES and ROSA (10) state:

The analyses of RAMSEY and ROBINSON (1917) show that carbohydrate, N, and ash constituents of the mother set are far from exhausted, even at the end of the growing season.

Still more recently we have the report of LUDWIG (11), which contains data on the composition of the mother tuber after growth in culture solutions. His experiments showed that after the sprouts had become 6, 8, or even 30 cm. high, with normal dark green leaves, and with roots and stolons 20 cm. long, the mother tubers still retained 40 per cent of their total nitrogen and about 65 per cent of their carbohydrate reserves. He emphasizes the "common observation" that eyes dug out of tubers will produce normal plants with tubers not inferior to those produced by seed tubers planted in the usual way. He states that, during the late war, farmers used not only pieces of tubers, but also merely well developed eyes attached to pieces of potato peelings, and these gave normal yields. The failures that had resulted from the use of cut tubers were thought to be due, not to the small quantity of reserve food in the seed-pieces, but to loss by rotting.

DE VRIES (6) studied the transfer of food reserves from the mother tuber to the sprout at different stages of growth. These observations were largely qualitative, no analyses being furnished and no amputation experiments being carried out. His opinion was that the mother tuber furnished nutrients to the sprout until germination was completed (plants above ground with green leaves), but that after the beginning of formation of young stolons the food supply was thenceforth transferred only to the newly forming tubers.

<sup>3</sup> One paragraph in MÜLLER's article is significant in connection with the present experiments. He states: "It would be interesting to carry out a special experiment to determine in what manner the absolute weight of the mother tuber changes during the vegetative period. Also, how the yield of young tubers would be influenced by removing the mother tuber after the sprout had completely developed."

Although, as will be shown later, the evidence is in favor of the older view that the depletion of the food reserves stored in the mother tuber is extensive or nearly complete, there is no agreement as to the nature of the substances furnished by the mother tuber that are so influential in increasing growth. APPLEMAN (1), by a series of experiments on the relation between the size of the seed-pieces and the strength of the sprout, showed that when the size of the seed-piece fell below a certain minimum, the vigor of the sprout decreased as the size of the seed-piece was reduced. He believed that the failure of small pieces to produce good sprouts was not due primarily to the lack of usual food materials, "as sprouts on pieces still large enough to contain an abundance of these substances show considerable decrease in vigor." He concluded "that the potato tuber contains a limited amount of a special growth promoting substance, and if the amount of tissue surrounding the growing bud is too small there is not enough of this substance available for normal growth." The view expressed by JONES and ROSA (10) is that "the large set furnishes something to the plant arising from it, which is not present in sufficient amount in the smaller set."

From this short summary of the literature, it is apparent that further information is needed upon the subject. In previous amputation experiments either not enough plants have been used, or not enough stages of development of the plants have been included in the test to give a complete account of the relation of the mother tuber to the sprout. The observations on the effect of the removal of mother tubers have not been accompanied by chemical analyses of the amputated tissue. The data as to the extent of the utilization of the mother tuber show sharp disagreements, and further analyses are needed to show which of these divergent views is correct. The present experiments have provided additional information upon several of the questions involved, but a discussion as to the bearing of the results upon various phases of the problem will be postponed until the experimental results themselves have been described.

### Results

The experimental results may be divided into two parts, the first dealing with the amputation of the mother tubers at intervals after planting and its effect upon subsequent growth of the plant, the

second dealing with the chemical composition of the mother tissue that was removed at different periods of growth.

# AMPUTATION OF MOTHER TUBER AND ITS EFFECT

METHOD OF REMOVAL FROM SPROUT.—Since it was essential that the mother tuber be removed from the sprout with as little disturb-



FIG. 1.—Method of amputating mother tuber from sprout: left, plant grown in pot buried in soil; right, plant inverted, placed upon board with slot from edge to center used for support; pot then discarded. Note mother tissue being removed piece by piece with scalpel; plant placed in soil after removal of mother tuber.

ance of the root system as possible, the plants were grown in clay pots which were planted in the soil, the top of the pot being slightly below the surface of the soil. At any subsequent period of growth at which it was desired that the mother tuber be removed, a number of pots containing the now well-rooted plants were removed from the soil and inverted (fig. 1). The mother tuber, which had been

planted in the bottom of the pot just above the drainage hole, was now freely exposed and could easily be detached by cutting; the pot was then discarded and the plant was replaced in the soil, and from this time onward grew under normal conditions, that is, undisturbed by the restrictions of growth in pots.

At each stage of development at which mother tubers were removed from a certain number of plants, an equal number of plants were removed from pots and planted in the soil, but from these the mother tubers were not removed. These were considered as check plants, since they were handled in precisely the same way except with reference to the removal of mother tubers. It is true that there would be somewhat greater disturbance of the soil in removing the mother tubers than would result in handling the checks, but an attempt was made to equalize this difference partially, by purposely disturbing the soil around the mother tubers in the check to about the same degree.

In setting the plants in the soil, the arrangement of the amputated plants and their corresponding checks depended upon the stage of development at which the mother tubers were removed. When they were amputated at early stages, for example, at stages 1 and 2 (fig. 2), the plants were arranged in the field in sets containing five plants each, that is, five plants of the amputated series, then five plants of the check series, then five plants of the amputated series, etc. At the later stages of growth, for example, at stages 3 and 4 (fig. 2), however, the amputated plant and the corresponding check were planted side by side, in order to equalize soil differences as nearly as possible and to permit more dependable comparisons.

It was found that the plan of having a set of checks for each set of plants from which mother tubers were removed, and repeating this at each stage of growth at which they were removed, was essential in order to permit dependable comparisons at the end of the season. In the first year of the experiments (1926) this precaution was not taken, the "checks" being merely plants which had never been in pots at any part of the season and from which mother tubers were not removed. The yield data at the end of the season did not therefore take into account the influence of the growth in pots, as compared with growth in free soil, up to the time of removal of

mother tubers. Consequently the yield data for 1926 had to be rejected. In 1927 and 1928, however, the amputated lots and the corresponding check lots grew for the same periods in the same sized pots, and for the same periods in the soil, the only variable factor being presence and absence of the mother tuber.



FIG. 2.—Conditions of plants at four different stages at which mother tubers were amputated: A, stage 1; B, stage 2; C, stage 3; D, stage 4.

The preliminary experiments in 1926 also showed that the exact method of removing the mother tuber from the plant was a matter of considerable importance. When it was removed by cutting across the stem at the surface of the tuber, or by forcibly twisting it off from the stem, bleeding at the cut end of the stem occurred. This often resulted in wilting of the plant, even when the well-rooted plant with undisturbed root system was placed at once in soil to

which an abundant amount of water had been added. This difficulty was obviated in 1927 and 1928 by sectioning off small portions of tissue from the mother tuber piece by piece, until finally a small piece of it was allowed to remain at the base of the stem. Under these conditions no wilting was observed. The method of removing the mother tuber is shown in fig. 1, and the condition at the base of the stem after amputation was completed is shown in fig. 4 (see left-hand plant at K).

SIZE OF SEED-PIECE AND VARIETIES.—Seed-pieces weighing 28 gm. each were used, and in order to be assured of uniformity in this respect the pieces were weighed individually, and trimmed until the weight did not vary by more than 1 gm. In the 1926 and 1927 experiments only Irish Cobbler variety was used, but in the 1928 work Bliss Triumph was included in the main experiment. In this year also, a preliminary test was made with a number of other varieties, as shown in table VI, in order to note the behavior of various varieties with respect to utilization of the dry matter in the mother tuber.

SIZE OF POTS.—For the series in which the mother tubers were to be removed at stage 1 (fig. 2), clay pots 3.5 inches in diameter and 3.5 inches deep were used; for the series at stage 2 the pots were 6 inches by 4.75 inches; for the series at stage 3 they were 8 inches by 4.5 inches; and for stage 4 two sizes were used, 10 inches by 4.5 inches and 12 inches by 4.5 inches.

STAGES OF DEVELOPMENT AT WHICH MOTHER TUBERS WERE REMOVED.—The mother tubers were removed at four different stages of development, and the sizes of the plants at each amputation stage are shown in fig. 2. At stage 1 (A) the mother tubers were removed when the sprouts had just pushed through the soil. The tips were green, but the young leaves had not yet expanded. This was the condition 22 days after planting. At stage 2 (B) the mother tubers were removed when the sprouts were about 2 inches above the surface of the soil and the leaves fully expanded. This was the condition of the plants 29 days after planting. At stage 3 (C) the plants were about 10 inches above the surface of the soil, were making a vigorous growth, underground stolons several inches long had formed, and young tubers were well started in development. These plants might be termed half-grown. This was the condition 42 days after planting.





FIG. 3.—History of development of amputated and check plants during growth period when mother tuber was removed at stage 1: *E*, amputated (left) and check (right) at time of amputation, May 24; *F*, two plants of same series showing condition of amputated and check plants on June 8; *G*, further stage in growth of amputated (left) and check (right) plants as shown by representative plants on June 19; *H*, comparison of amputated (left) and check (right) plants on July 31.

At stage 4 (D) the plants were about 24 inches above the surface of the soil, had attained approximately the maximum height of vine, were in full bloom, and many young tubers were fully formed. This was the condition 56 days after planting.

**SUBSEQUENT GROWTH OF PLANTS AFTER AMPUTATION.**—The growth of the plants from which the mother tubers were removed as compared with the growth of the corresponding check plants is shown in figs. 3 and 4. These photographs were obtained by selecting what appeared to be average plants from each group, and placing the treated and check plant side by side for photographing. Because of the great variability of potato plants it will be recognized that only rough comparisons can be obtained in this way, since a single plant cannot be depended upon to show accurately the average condition of a group.

The subsequent growth of the plants from which mother tubers were removed at stage 1 is shown in fig. 3. The condition of the amputated plant and its check on the day of the removal of the mother tuber (May 24) is shown at E; and F, G, and H show the conditions of the plants on June 8, June 19, and July 31 respectively. It will be observed that the plants from which the mother tubers were removed made slow growth at first, but that later in the season a surprisingly large amount of vine was developed. The amputated plant shown in fig. 3 (H, left) shows a comparatively larger quantity of tubers, however, than is justified by the final yield data shown in table IB, thus indicating that although the plant used for the photograph showed an average condition of vine, the underground condition was not truly representative of the group. At any rate it is apparent that removing the mother tubers at this early stage influenced both the subsequent vine growth and tuber yield, and that the effect was greater upon the yield of tubers than upon the final size of vine.

The subsequent growth of the plants from which mother tubers were removed at stage 2 is shown in fig. 4. The condition of the amputated plant and its check (May 31) is shown at K, and the condition on June 19 is shown at L. From this date onward the amputated plants grew rapidly enough to overtake the checks, so that later in the season no difference in size of vine could be observed between amputated and check plants, and consequently no further photo-

graph in this series was made. As for the plants at stages 3 and 4, the condition of the checks is shown in fig. 2, C and D. The subsequent rates of growth of the vines by amputated and check plants in these two series were so nearly equal that no difference was observable and further photographs were not made. But, although the amputation at these stages did not produce an observable effect upon



FIG. 4.—K, condition of amputated (left) and check (right) plants at time of amputation (May 31) when mother tubers were removed at stage 2; L, representative samples of same series on June 19; later in season the vine growth of amputated and check plants approximately equal.

the growth of vines, it will be seen from the data in table IB that it is possible that the yield of tubers was influenced, either unfavorably as in the case of Irish Cobbler, or favorably as in the case of Bliss Triumph.

**YIELDS OBTAINED.**—Although a yield record was obtained in the 1926 experiments, these data are not included in this report because of the failure to obtain adequate checks, as described in a previous paragraph. The plants in the 1927 experiments suffered from attacks of aphids, and the growth of many of the plants from midseason until the vines died was not normal, many plants dying and other plants

being obviously injured by the numerous spray applications that were needed. This resulted in many missing hills in the field; and, since in these experiments the arrangement of the plants in the field was a necessary feature in permitting dependable comparisons as to the final yield of amputated and check plants, the yield data for 1927 were excluded. In the 1928 experiment (which is to be considered the main one in this series), however, the conditions were favorable in all respects for normal growth; and, since the amputated lots had comparable checks in each case, the data are considered reliable and are presented in tables IA and IB.

In table IA the first four columns show the yield in grams obtained from plants from which the mother tubers were removed at stage 1, that is, when the sprouts were first emerging from the soil. Columns 1 and 2 show the results with Irish Cobbler, and columns 3 and 4 show those with Bliss Triumph. Each figure represents the yield in grams from a single plant. The average yields when mother tubers were not removed were 255 gm. for Irish Cobbler and 350 gm. for Bliss Triumph, while the yields per plant when mother tubers were removed were 86 gm. for Irish Cobbler and 113 gm. for Bliss Triumph. Table IA, columns 1, 2, 3, and 4 show that removing the mother tubers at the time of emergence of the sprout markedly reduced the yield. Furthermore, it will be noted (columns 2 and 4) that many of the plants from which mother tubers were removed at this early stage failed to develop at all. At this period of development the presence of the mother tuber is critical.

The yield data for stage 2 are shown in table IA, columns 5, 6, 7, and 8. Here again each figure represents the yield from a single plant. Columns 5 and 6 show that the average yield for Irish Cobbler was  $328 \pm 12.2$  when the mother tuber was left on, and  $274 \pm 9.3$  when it was removed. The difference (54 gm. per plant) is 3.5 times its probable error, and is therefore significant, showing that the removal of the mother tuber at this stage reduced the yield. In the case of the Bliss Triumph (columns 7 and 8) the corresponding values are  $244 \pm 11.4$  for plants with mother tubers left on, and  $197 \pm 11.8$  for plants with them removed. The difference (47 gm. per plant) is only 2.86 times its probable error, and is insufficient to show that amputation reduced the yield. Further experiments with this varie-

ty at this stage of development are necessary. It is apparent that the planting plan must be such as to place the amputated plants and their corresponding checks side by side in the field, in order to permit

TABLE IA  
EFFECT OF AMPUTATION OF MOTHER TUBERS UPON YIELD

MOTHER TUBERS REMOVED AT TIME OF EMERGENCE OF SPROUT (STAGE 1, FIG. 2A)				MOTHER TUBERS REMOVED AFTER GERMINATION WAS COMPLETE AND LEAVES UNFOLDED (STAGE 2, FIG. 2B)			
IRISH COBBLER		BLISS TRIUMPH		IRISH COBBLER		BLISS TRIUMPH	
Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.
115	40	493	59	449	358	370	258
255	46	198	118	527	202	357	94
350	40	102	140	385	282	385	190
343	75	418	42	447	290	260	109
470	35	474	74	*	*	*	120
292	27	403	80	257	390	115	330
222	25	275	*	468	270	190	155
215	26	177	*	268	276	207	243
290	*	354	*	170	290	170	257
143	*	253	*	240	248	107	225
430	105	307	*	277	294	270	44
235	208	465	*	294	295	252	29
230	*	454	*	257	360	329	62
85	*	193	*	380	199	208	85
129	*	330	*	303	320	240	190
288	120	318	230	306	440	440	185
287	75	303	42	520	440	207	285
355	97	615	72	340	330	170	285
273	*	192	185	370	415	472	300
75	160	329	146	520	240	180	*
203	44	474	172	340	362	390	192
318	145	490	126	209	290	235	375
270	150	*	98	228	192	250	176
*	128			195	290	172	115
Average 255	Average 86	Average 350	Average 113	256	190	290	390
				302	100	70	250
				308	222	190	202
				153	279	185	70
				162	142	308	*
				274	147	*	*
				340	247	135	77
				330	173	290	310
				347	244	123	270
				456	241	215	135
				390	257	254	304
				Average 328	Average 274	Average 244	Average 197
				P.E. $\pm 12.2$	P.E. $\pm 9.3$	P.E. $\pm 11.4$	P.E. $\pm 11.8$

\* Plants failed to develop; missing hills not included in computing averages and probable errors.

more critical comparisons regarding the influence of amputation at this stage upon yield.

The yields for the series in which the mother tubers were removed at stage 3 (when plants were half-grown) and stage 4 (when plants had attained maximum height and were in bloom) are shown in

table IB. Since the two varieties behaved quite differently with respect to the effect of the removal of mother tubers at these two stages, they will be discussed separately. The results with Irish Cob-

TABLE IB  
EFFECT OF AMPUTATION OF MOTHER TUBERS UPON YIELD  
(CONTINUATION OF TABLE IA)

MOTHER TUBERS REMOVED WHEN PLANTS WERE 10 INCHES HIGH, AND YOUNG TUBERS FORMING (STAGE 3, FIG. 2C)				MOTHER TUBERS REMOVED WHEN PLANTS WERE NEARLY MAXIMUM HEIGHT, AND IN BLOOM (STAGE 4, FIG. 2D)			
IRISH COBBLER		BLISS TRIUMPH		IRISH COBBLER		BLISS TRIUMPH*	
Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.	Not removed, yield in gm.	Removed, yield in gm.
200	105	135	253	130	154	85	200
160	154	140	170	117	30	58	68
192	290	122	125	106	47	106	124
59	130	242	200	183	146	182	125
58	200	202	208	125	180	125	155
140	177	150	249	134	225	142	95
402	226	302	275	102	187	232	198
193	160	186	253	247	107	138	116
222	290	333	204	251	190	140	167
270	265	274	202	118	155	106	196
240	170	116	180	173	204	90	180
250	70	106	366	155	170	148	190
140	254	150	144	135	130	210	234
253	215	176	294	196	142	116	154
150	196	78	157	103	103	160	225
223	165	127	201	112	117		
190	144	252	93	103	52	Average 140	Average 162
206	172	156	115	56	111		
220	198	135	89	136	216		
308	70	60	106	200	172		
268	142	256	240	204	118		
290	120	146	153	183	216		
126	203	152	180	189	207		
235	190	40	250	177	255		
224	150	198	150	311	280		
190	135	103	120	177	230		
100	102	69	53	126	208		
120	70	142	233	235	197		
174	90	173	56	178	210		
120	90	131	121	218	154		
190	108	117	99	157	172		
192	122	133	107	202	218		
148	130	181	212	158	147		
125	146	90	156	230	223		
190	220	125	159	187	253		
153	190	128	206	253	186		
73	202	44	176	162	202		
240	127	140	220	138	155		
130	207	161	163	110	165		
185	75			133	112		
170	176	Average 153	Average 181	88	206		
204	206			228	212		
243	148			105	135		
155	190			179	62		
170	277			45	78		
				102	109		
Average 189	Average 166			Average 160	Average 164		

\* Because of rotting of mother tubers of Bliss Triumph between stages 3 and 4, only 30 plants with firm mother tubers were available for amputation at this stage.

bler for stage 3 are shown in columns 1 and 2, table IB, and show the response from 45 pairs of hills, one plant of each pair being the check from which the mother tuber was not removed, and the other being the plant from which the mother tuber was removed at stage 3 (plants half-grown, fig. 2C). The average yield per plant when mother tubers were left on was 189 gm., and that for plants from which mother tubers were removed was 166 gm.

It was anticipated that probably only small differences would be found in the yields from plants with mother tubers not removed, as compared with plants from which they were amputated at these later stages of growth. Consequently, in transplanting after removal of mother tubers in the two series at stages 3 and 4, the amputated and check plants were placed adjacent to each other in pairs, in order that the soil conditions would be more nearly alike, and in order that the yield data could be compared by the simultaneous difference method employed by ENGLEDOW and YULE (7) and by use of the formula given by FISHER (8). Calculations made by ENGLEDOW and YULE's method show odds of only 13 to 1 for significance of the observed difference in this case. When the FISHER formula is applied to the same data, the value of "t" found was 1.77, which, as shown by the table for "t" values (FISHER 8, p. 137), corresponds to odds of less than 20 to 1. FISHER's requirement for a probability of  $P = 0.05$  would necessitate a value of about 2.0 in this case; the value actually found (1.77), therefore, falling short.

Taking into account the probabilities shown by these two methods, it seems that, while there is some evidence that the removal of mother tubers decreased the yield, the assurance is not complete, and further experiments must be made to permit a definite statement as to the influence of removal at this stage of development.

The response of Irish Cobblers to the amputation of mother tubers at stage 4 (plants nearly full height and in bloom) is shown in table IB, columns 5 and 6. In this case the average yield with mother tubers left on was 160 gm. per plant, and with them removed was 164 gm. Removing the mother tubers at this late stage of development had no effect upon the final yield of tubers.

The results obtained with Bliss Triumph variety at stages 3 and 4 are shown in columns 3, 4, 7, and 8 in table IB. In examining these

data a surprising result is obtained, namely, the plants from which the mother tubers were amputated gave larger yields than those from which they were not removed.

Thus (columns 3 and 4, table IB) the yield from plants from which the mother tubers were removed at stage 3 (half-grown) was 181 gm. per plant, while the yield from plants from which they were not removed was 153 gm. There were thirty-nine pairs of plants available for this comparison. When the data are treated by the method of ENGLENDOW and YULE (7), it is found that the odds are 36 to 1 that the observed difference is significant. When use is made of the formula of FISHER (8), the value of "t" resulting from the calculation is found to be 2.42. When this value is fitted into FISHER's table of "t" values it is found that his requirement of a probability at least equal to  $P=0.05$  has been satisfied, and that odds greater than 19 to 1 in favor of the view that the amputated series has given greater yields than the checks have been obtained.

A probable cause of this result may be found in the fact that, in the period following stage 3, the Bliss Triumph mother tubers that remained in contact with the sprout began to rot badly. When the time arrived to amputate them at stage 4, most of the plants had to be discarded because, upon inverting the pots, it was found that the mother tubers were either partly or completely rotten. Therefore, only thirty plants with firm mother tubers were available for the comparison at stage 4. The results of the amputation at stage 4 are shown in table IB, columns 7 and 8. Here again a greater average yield was found for the plants from which mother tubers were removed, that is, 162 for the amputated and 140 for the corresponding checks. STUDENT's (19) method shows odds of 19.5 to 1 that this difference is significant, but the calculation by FISHER's method gives a "t" value for the observed data amounting to 1.84, which is somewhat below the "t" value (about 2.0) that would be required by him for a probability of  $P=0.05$ , that is, 19 to 1. Thus, while the data partially support the view that contact with the mother tuber at this stage of development has reduced the yield of tubers, the odds are not sufficient to give assurance. The evidence at stage 4, however, tends to substantiate the similar condition found at stage 3.



It will be noted in tables IA and IB, that the check plants from which mother tubers were not removed gave yields that differed considerably in the four series. Thus the checks for the amputation at stage 1 gave an average yield for Irish Cobbler of 255 gm. (table IA, column 1); the checks for stage 2 yielded 328 gm. per plant (table IA, column 5); while for stages 3 and 4 the yields of check plants were 189 and 160 gm. respectively (table IB, columns 1 and 5). Since these were check plants (mother tubers not removed), amputation could not have been a factor, yet the differences in yield were even greater than the differences due to amputation. It should be remembered that the check plants grew in pots for different lengths of time in the four series, and that after amputation the plants were of necessity transplanted into different parts of the field in the four series. Consequently the conditions for growth were considerably different, and a difference in yield could be expected. These differences between the checks in the different series, however, do not vitiate the differences between the amputated and check plants in each series, since the experiment was arranged to make conditions within each series the same for both amputated and check, except for presence and absence of mother tubers. The high variability in yield of individual potato plants, and the differences observed between checks in the four series, emphasize the importance of the precautions taken to arrange the experiment so that the amputated plants and their corresponding checks could be compared directly with each other, under conditions that make these disturbing factors inoperative.

#### CHEMICAL COMPOSITION OF AMPUTATED MOTHER TUBERS

Analyses of the tissue obtained in amputating mother tubers at intervals after planting were obtained for all three years. Although, as previously described, the yield data were not suitable for comparisons in the 1926 and 1927 experiments, because of inadequate checks or interference with the planting plan after amputation, the plants from which mother tubers were removed had made apparently a normal growth up to the time of amputation, and consequently the analytical results from the amputated tissue should be of value, and are included.

**SAMPLING METHODS.**—As soon as the potato tissue was removed from the sprout the pieces were wiped with a moist rag, and peeled to remove the old epidermis and the suberized layer that had formed at the cut surface. The tissue was then either minced in a food grinder or chopped in a bowl. In the preliminary experiments in 1926 and 1927 it was found that, as the season progressed, the mother tubers became very high in water content; hence when the tissue was passed through a food grinder liquid was pressed out, and it was difficult to get a sample of the tissue which represented uniform proportions of solid and liquid. In the 1928 experiments, therefore, the potato tissue was chopped into fine pieces in a wooden bowl, and better sampling obtained by this method. As soon as the tissue was ready for sampling, weighed portions were removed for the moisture determination. To obtain a sample for the subsequent analyses for all constituents except sugar, weighed amounts (150–300 gm. per sample) were dropped (small quantities at a time) into boiling alcohol. The amount of alcohol used was adjusted so that the final concentration, taking into account the water content of the tissue, would be about 70 per cent. The tissue was stored in this concentration, but when the extractions for the analyses were begun, water was added to make the alcoholic concentration 50 per cent by volume. Three extractions at boiling temperatures were made, the liquid being decanted after settling. The tissue was then dried and ground to a fine powder which was extracted twice in the same manner, the decanted portions being added to the liquid obtained by the first three extractions.

Fifty per cent alcohol (by volume) was selected as the solvent for two purposes: first, to separate the starch from non-starch substances, as recommended by BRYAN, GIVEN, and STRAUGHN (4); and second, to separate protein from non-protein nitrogen. Working with the alfalfa plant, OSBORNE, WAKEMAN, and LEAVENWORTH (13) found that alcohol at 53 per cent (by weight) precipitated protein completely from the juice, and that 20 per cent (by weight) caused the precipitation of most of the protein present. In the present experiments it was found that very little if any protein was extracted by alcohol at a concentration of 50 per cent (by volume). Tests by such protein precipitants as colloidal iron, trichloroacetic acid, and

tannin produced small precipitates in which only traces of nitrogen were present. Lead acetate precipitated appreciable quantities of nitrogen, but the results of VICKERY and VINSON (22) indicate the possibility that these were substances other than protein.

The extractions were made up to a definite volume after cooling, and this liquid was centrifuged, decanted, and aliquots taken for analysis. This was called the soluble portion, solubility in this case referring to 50 per cent alcohol (by volume) as the solvent.

The small amount of precipitate obtained in centrifuging the extract was added to residue from the extraction, and this, which was called the insoluble portion, was dried on a water bath and ground to a fine powder.

Separate samples of the fresh tissue were taken for the sugar determinations, and this tissue was also dropped into boiling alcohol, exactly as just described for the main sample except that calcium carbonate was added to the alcohol.

METHODS OF ANALYSIS.—For reducing sugars the MUNSON and WALKER method (2) was used, and the precipitated cuprous oxide was titrated with potassium permanganate, the permanganate value being determined by comparison with a sample of dextrose obtained from the Bureau of Standards. Sucrose was determined by inverting in the cold with hydrochloric acid (2). Ammonia was estimated by the aeration method after adjusting the solution to slight alkalinity. The apparatus of VAN SLYKE and CULLEN (21) was used and the aeration period was 2.5 hours. For amide, the liquid after ammonia aeration was neutralized with a drop or two of acid, and, after the addition of 1 cc. of concentrated hydrochloric acid for each 10 cc. of liquid, was hydrolyzed for 1.5 hours; the liquid was then transferred to an evaporating dish and the hydrochloric acid removed by evaporation. The liquid was then made slightly alkaline and again aerated in the VAN SLYKE-CULLEN apparatus, and the ammonia formed during the hydrolysis was estimated. For "basic" nitrogen, the liquid remaining after the amide aeration was used; phosphotungstic and sulphuric acids were added, and the liquid was heated to boiling. It was then cooled, made up to volume in a flask, mixed thoroughly, and allowed to stand over night. The precipitate was separated by the use of the centrifuge; to it were added the particles

of precipitate that remained in the volumetric flask, and the combined precipitates were washed twice with dilute phosphotungstic acid solution. The final precipitate was analyzed for nitrogen by the KJELDAHL method (2). The liquid decanted from the phosphotungstic precipitate was used for both the non-basic and amino-acid nitrogen, the non-basic by the KJELDAHL method, and the amino-acid gasometrically by the VAN SLYKE procedure (20). For the estimation of starch the diastase method with subsequent acid hydrolysis was used (2), but in this case saliva was found preferable to the malt extract because of the low "blank" value with saliva.

RESULTS OF ANALYSES.—The percentage composition calculated on the basis of fresh weight of the tissue is shown in table II and fig. 5. It will be noted that a rapid fall occurred in the percentages of all constituents except moisture, sugars, and soluble solids. Thus while the starch percentage dropped from about 10-15 to about 0.3-0.6 per cent (table II, column 8), the water content increased from about 70-80 to about 96 per cent. The various forms of nitrogen (table II, columns 11-17) all show a continuous reduction in percentage composition throughout the period of analysis, until finally the mother tubers removed at stage 4 were almost completely depleted of all forms of nitrogen, the percentage remaining being only about one-fifth or one-tenth of that present in the mother tubers at the time of planting. The change in the percentage of reducing sugar shows an interesting behavior in that, although there is a continuous loss of dry substance, the percentage of reducing sugar actually increased (table II, column 9). The details of these changes vary somewhat with the variety and with different years. There is a tendency for the percentage to be highest in midseason, that is, when the plants are about 10 inches high, and to recede again at the later stages. Another interesting feature is to be noted with respect to the percentage of solids soluble in 50 per cent alcohol. This tends to remain more nearly constant than any other constituent for which data were obtained. Thus, up to about stage 3 (stage 2 in the case of Bliss Triumph in 1928) the percentage of soluble solids was about 80 per cent of the value at the start of the experiment. Probably the increase in sugar explains this maintenance of a high soluble solids value in spite of the continuous loss of dry weight during the

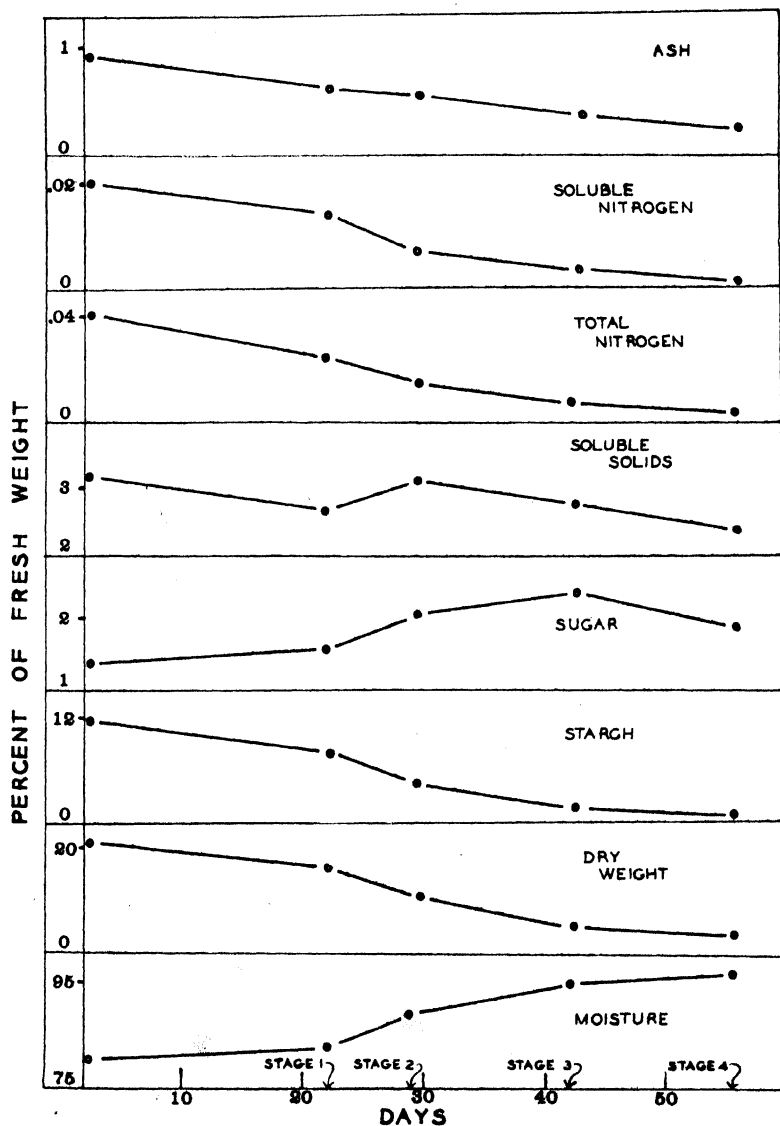


FIG. 5.—Composition of mother tuber tissue at beginning of experiment and at amputation at different stages of growth: points in circles over abscissa at 22 days show composition of mother tuber tissue removed at stage 1, those over the 29-day abscissa at stage 2, etc.; ordinates show percentages on fresh weight basis. Note change in percentage range for each constituent (left) to correspond to requirements of different constituents.

TABLE II

CHEMICAL COMPOSITION OF MOTHER TUBERS REMOVED FROM POTATO SPROUTS AT DIFFERENT INTERVALS AFTER PLANTING

VARIETY AND DATE OF PLANTING	DATA REGARDING REMOVAL OF MOTHER TUBERS				PERCENTAGE COMPOSITION ON FRESH WEIGHT BASIS											
	Date of removal	No. days after planting	No. days after emergence of sprout	Condition of plant at time of removal	H <sub>2</sub> O	Solids soluble in 95% alcohol	Starch	Re- ducing sugars	Cane sugar	Nitrogen (N)						
										Insoluble in 95% alcohol	Soluble in 95% alcohol	NH <sub>3</sub> N	Amide N	Basic N	Non- basic N	Amino N
Irish Cobbler, May 12, 1926	Seed-pieces at start, before			planting	71.6	2.80	15.72	0.55	0.15	0.17	0.40	0.033	0.014	0.087	.....	0.078
	June 4 23	2			87.6	2.51	6.15	0.99	0.11	0.06	0.27	0.027	0.009	0.027	.....	0.051
	June 14 33	12			91.8	2.62	3.05	1.88	0.08	0.04	0.20	0.012	0.010	0.031	.....	0.044
	June 21 40	19			94.3	2.41	1.41	1.93	0.12	0.03	0.11	0.011	0.005	0.026	.....	0.024
	June 29 48	27			96.0	2.11	0.48	1.01	0.15	0.02	0.08	0.003	0.002	0.021	.....	0.027
Irish Cobbler, April 22, 1927	Seed-pieces at start, before			planting	80.4	2.81	10.61	0.50	0.16	0.13	0.21	0.017	0.023	0.076	.....	0.096
	May 28 36	7		Sprouts 2" high	89.3	2.35	3.82	1.28	0.50	0.08	0.07	0.007	0.010	0.027	.....	0.039
	June 10 55	20		Sprouts 9" high	93.3	2.04	0.88	1.42	0.44	0.04	0.03	0.002	0.005	0.019	.....	0.015
	June 30 69	40		Sprouts 13" high	95.3	1.70	0.60	1.43	0.27	0.03	0.01	0.001	0.002	0.025	.....	0.009
	Seed-pieces at start, before			planting	78.00	3.17	11.80	0.89	0.46	0.21	0.20	0.008	0.036	0.040	0.100	0.084
Irish Cobbler, May 2, 1928	May 24 22	0		Emergence of sprouts (stage 1, fig. 2A)	83.70	2.93	8.15	1.20	0.40	0.11	0.15	0.005	0.019	0.050	0.060	0.047
	May 31 29	7		About 2" high, leaves expanded (stage 2, fig. 2B)	88.60	3.15	4.89	1.42	0.72	0.07	0.08	0.005	0.009	0.030	0.035	0.025
	June 11 42	20		About 10" above ground, half-grown (stage 3, fig. 2C)	94.40	2.82	1.09	2.02	0.42	0.03	0.04	0.002	0.006	0.014	0.014	0.015
	June 25 56	34		Maximum height, in bloom (stage 4, fig. 2D)	96.00	2.27	0.30	1.61	0.21	0.02	0.02	0.001	0.003	0.007	0.006	0.008
	Seed-pieces at start, before			planting	81.72	3.66	9.55	1.11	0.63	0.11	0.17	0.007	0.026	0.042	0.071	0.054
Bliss Triumph, May 2, 1928	May 24 22	0		Emergence of sprouts (stage 1, fig. 2A)	85.25	2.87	7.93	1.24	0.29	0.10	0.11	0.004	0.014	0.030	0.044	0.028
	May 31 29	7		About 2" high, leaves expanded (stage 2, fig. 2B)	91.90	3.35	3.33	1.86	0.46	0.06	0.05	0.002	0.007	0.013	0.018	0.014
	June 11 42	20		About 10" above ground, half-grown (stage 3, fig. 2C)	96.40	2.22	0.40	1.40	0.49	0.03	0.02	0.002	0.003	0.007	0.007	0.007

same period. Later in the season the percentage of soluble solids dropped, but even at stage 4 its value was found to be 60-70 per cent of the original value.

The ash analyses, given in table III, are for the 1928 series only. Columns 2 and 5 show the percentage of ash obtained by ashing the portion of the tissue that was insoluble in 50 per cent alcohol; columns 3 and 6 show the ash percentages obtained by ashing aliquots of the liquid extract; and columns 4 and 7 represent the total ash obtained by adding the soluble and insoluble percentages. These analyses show a gradual fall in the different forms and in the total ash from the beginning to the end of the experiment. The ash,

TABLE III

ASH IN MOTHER TUBER TISSUE AMPUTATED FROM SPROUTS AT DIFFERENT PERIODS OF GROWTH

STAGE OF GROWTH AT TIME OF AMPUTATION	PERCENTAGE ON FRESH WEIGHT BASIS					
	IRISH COBBLER			BLISS TRIUMPH		
	Insoluble*	Soluble*	Total	Insoluble*	Soluble*	Total
Start of experiment.....	0.425	0.455	0.880	0.285	0.316	0.601
Stage 1 (fig. 2A).....	0.337	0.368	0.705	0.273	0.300	0.573
Stage 2 (fig. 2B).....	0.302	0.310	0.612	0.195	0.197	0.392
Stage 3 (fig. 2C).....	0.208	0.195	0.403	0.167	0.128	0.295
Stage 4 (fig. 2D).....	0.020	0.160	0.180	†	†	†

\* Percentage ash in the portion of tissue either soluble or not soluble in 50 per cent alcohol (by volume)

† Tissue not available for analysis (see text).

however, was not used up as completely as the dry weight or various forms of nitrogen, as shown in the previous paragraphs. Table III shows that at stage 3 approximately one-half of the ash was still present in the mother tissue. An analysis of the ash for various constituents was not made, but the ash has been retained for a subsequent analysis. The question as to the rate of utilization of the different ions is an interesting one, made especially so by the observations of MÜLLER (12) and of RAMSEY and ROBERTSON (14). In both contributions it is reported that potassium remains in the mother tuber and does not take part in the growth of the sprout.

CHANGES IN FRESH AND DRY WEIGHT OF INDIVIDUAL MOTHER TUBERS.—Since the seed-piece loses dry substance to the sprout, and also of course loses substance by its own respiration, and at the same

time gains in water content, it may be inquired whether, as a result of these two opposing factors, the absolute weight of the mother tuber will increase or decrease during the period of the experiment.

In the 1926 and 1927 experiments, no account was taken of the absolute change in weight of the mother tuber during the season. Hence, although the data could be calculated upon either the fresh or dry weight basis, it was impossible to determine the absolute amounts of materials leaving the individual mother tuber at any stage. In 1928 a special experiment was carried out to obtain data on this point (table IV). About 75 seed-pieces of each variety were carefully weighed on a small balance in order to obtain a weight of 28 gm. Ten of these were weighed at once, and the average weight was found to be 28.20 gm. for Irish Cobbler and 28.25 gm. for Bliss Triumph (table IV). The average deviation of the weight of any one seed-piece from the average weight of the ten was about one per cent. The other sixty-five pieces of each variety were then planted in the soil, and permitted to grow until the plants arrived at the various stages of development at which mother tubers were amputated in the main experiment. At each stage ten plants were lifted, the mother tubers removed, and after careful wiping with a moist cloth, the fresh and dry weight of each was obtained. The average values are shown in table IV. The changes in fresh weight of the individual mother tubers are shown in columns 2 and 3. It is seen that the absolute weight did not change much throughout the season, the Irish Cobblers losing in weight from 28.20 to 26.22 gm., and the Bliss Triumph mother tubers gaining in weight from 28.25 to 29.02 gm. The dry weight and water content per mother tuber are expressed in grams in columns 4, 5, 6, and 7 in table IV. Thus, while each Irish Cobbler seed-piece lost 5.24 gm. of dry matter (that is, from 6.43 to 1.19 gm.), it gained 3.26 gm. of water (that is, from 21.77 to 25.03 gm.). This observation, that the absolute weight of the mother tuber did not change to any great degree during the season, facilitated the interpretation of the data obtained from the samples of tissue. It showed that calculations upon the fresh weight basis are capable of showing the true changes in composition with respect to the various constituents. And, although the observations showing that this is true were not made until 1928, it seems likely



that the 1926 and 1927 data can be evaluated safely from the same point of view.

In the last six columns in table IV the changes in fresh weight, dry weight, and water content of the mother tubers at each stage of development have been calculated from these data, and expressed as a percentage of the amount in the mother tuber at the time of planting. Special attention is directed to the large percentage losses in dry substance. About 25-30 per cent was lost by the time the sprout emerged from the soil, about 50 per cent at the time germination was complete, and about 80 per cent at the time the plant was half-grown. It is not maintained, of course, that all the dry substance lost by the mother tuber was gained by the sprout. It is certain that part of it was lost by the respiration of the mother tuber tissue, and some of it might merely have leached out into the soil.

#### ABSOLUTE AMOUNTS OF VARIOUS CHEMICAL CONSTITUENTS PER MOTHER TUBER

The data in table IV, showing the average fresh weight per mother tuber at each stage of development, combined with the data in table II, showing the percentage composition on the fresh weight basis, permitted a calculation of the absolute amounts of the various constituents in the individual mother tuber at each stage at which amputation was carried out. These data are shown for the 1928 experiments in table V.

By following down the columns in table V it is possible to observe the amounts of the various constituents present at the time of planting, and also the amount remaining in the mother tuber at each subsequent period of development.

One of the interesting features of table V relates to the utilization of starch between stages 2 and 3. At stage 2 leaves were fully formed, and the production of starch by the process of photosynthesis was going forward rapidly. It might have been expected that because of this there should have been less demand upon the stored starch, and yet during this period of high photosynthetic activity in the leaves the depletion of starch from the mother tuber was going forward at a rapid rate.

Another striking result was obtained with respect to the utiliza-

TABLE IV

FRESH WEIGHT, DRY SUBSTANCE, AND WATER CONTENT OF MOTHER TUBERS REMOVED FROM SPROUTS AT INTERVALS AFTER PLANTING

DESCRIPTION OF MOTHER TUBERS REMOVED FROM SPROUT	FRESH WEIGHT PER MOTHER TUBER (GM.)		DRY WEIGHT PER MOTHER TUBER (GM.)		WEIGHT OF H <sub>2</sub> O PER MOTHER TUBER (GM.)		PERCENTAGE OF AMOUNT IN MOTHER TUBER AT TIME OF PLANTING					
							Fresh weight		Dry weight		Water	
	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss	Cobbler	Bliss
As planted.....	28.20	28.25	6.43	5.47	21.77	22.78	100	100	100	100	100	100
Emergence of sprout (stage 1, fig. 2A).....	27.40	28.88	4.45	4.17	22.06	24.71	97	102	69	76	105	109
After germination complete and leaves expanded (stage 2, fig. 2B).....	27.46	29.24	3.20	2.79	24.26	26.45	97	104	50	50	111	116
Plants 10 inches high and young tubers starting to form (stage 3, fig. 2C).....	26.30	29.02	1.31	1.08	24.99	27.93	93	103	20	20	115	122
Plants in bloom and young tubers well developed (stage 4, fig. 2D).....	26.22	*	1.19	*	25.03	*	93	*	19	*	115	.....

\* Because of rotting of mother tubers of Bliss Triumph samples, a firm condition could not be obtained at this stage of development.

TABLE V

ABSOLUTE AMOUNTS OF SUBSTANCES PER MOTHER TUBER AFTER REMOVAL FROM SPROUTS AT INTERVALS AFTER PLANTING

CONDITION OF MOTHER TUBERS REMOVED FROM SPROUT	NITROGEN (N)												
	WATER (GM.)	SOLIDS SOLUBLE IN 50% ALCOHOL (GM.)	STARCH (GM.)	REDUCING SUGARS (GM.)	CANE SUGAR (GM.)	Insoluble in 50% alcohol (gm.)	Soluble in 50% alcohol (gm.)	NH <sub>3</sub> N (gm.)	Amide N (gm.)	Basic N (gm.)	Non-basic N (gm.)	Amino N (gm.)	
	Cob- bler	Bliss	Cob- bler	Bliss	Cob- bler	Bliss	Cob- bler	Bliss	Cob- bler	Bliss	Cob- bler	Bliss	
As planted.....	21.77122.779	0.894	1.034	3.328	2.698	0.251	0.314	0.130	0.178	0.059	0.031	0.056	0.0480.00210.00740.01130.01820.02010.02370.0153
Emergence of sprout (stage 1, fig. 2A).....	22.93824.717	0.803	0.835	2.233	2.290	0.329	0.358	0.110	0.084	0.039	0.041	0.0320.00140.00120.00520.00410.01350.00860.01640.01270.01290.0081	
After complete germination and leaves expanded (stage 2, fig. 2B).....	24.25626.450	0.865	0.981	1.343	0.974	0.390	0.544	0.198	0.135	0.019	0.017	0.022	0.0140.00060.00250.00200.00820.00380.00960.00520.00690.0040
Plants to inches high and young tubers starting to form (stage 3, fig. 2C).....	24.99027.935	0.742	0.644	0.287	0.116	0.531	0.406	0.110	0.116	0.008	0.009	0.011	0.0060.00050.00060.00160.00080.00370.00200.00370.00200.00390.0020
Plants in bloom and young tubers well developed (stage 4, fig. 2D).....	25.029	*	0.567	0.079	0.402	0.055	0.005	0.002	0.0006	0.0018	0.0015	0.0021	0.0021

\* At this stage of growth in the 1928 experiments, Bliss Triumph mother tubers were so badly rotted that a sufficient number could not be recovered.

tion of the sugars. Since the sugars are physiologically quite active, and therefore in condition for immediate use in respiration and growth, it might be expected that during periods of rapid growth these mobile substances would be used up rapidly and their concentration reduced to the minimum. Instead, however, the sugar actually increased during the period of rapid growth (see footnote 2). No doubt it was transported freely from the mother tuber, but this did not result in depletion, since apparently other compounds were broken down to sugar, and a high sugar level was maintained. The cane sugar situation is similar to that with reducing sugar, but the rate of loss of cane sugar was greater in the later stages of development (table V, columns 10 and 11).

The rate of disappearance of nitrogenous substances from the mother tubers is shown in table V, columns 12-25. The significant feature here is that all the forms of nitrogen for which analyses were made were utilized at about the same rate. It might reasonably have been expected that some forms would disappear faster than others, but the uniformity of the disappearance of these diverse groups is surprising. An estimate of this may be obtained by noting the number of cases in which the amount remaining at any stage is about one-half of that remaining at the previous stage. Out of forty-nine such pairs of values, thirty-one may be classed as essentially fulfilling this condition. Nothing of fundamental importance is claimed for this relation, which is quite accidental, since the stages chosen for amputation were merely empirical, but the coincidence serves to emphasize the facts observed. The data for 1926 and 1927 in table II also show a somewhat similar condition with respect to the rate of utilization of nitrogenous reserves.

#### BEHAVIOR OF DIFFERENT VARIETIES WITH RESPECT TO LOSS OF DRY WEIGHT

Because of the discovery of SELIBER (17) that the substance of the mother tuber of the variety Kruger is not utilized to any considerable extent by the sprout, a test was made with several varieties in order to see whether this behavior could be observed with American varieties. Tubers of ten varieties,<sup>4</sup> in addition to the Irish Cob-

<sup>4</sup> Appreciation is hereby expressed for the co-operation of Mr. WILLIAM STUART of the United States Department of Agriculture in supplying tubers of these varieties (except King Edward) for this experiment.

blers and Bliss Triumph used in the main experiment, were available for this test. Seed-pieces were prepared by first adjusting the fresh weights at approximately 20 gm. each, and then weighing them accurately on a balance. The average fresh weights are shown in table VI, column 2, and the corresponding dry weights in column 4. The seed-pieces were then planted and allowed to grow until the plants were about half-grown. The plants were then lifted, the mother tubers wiped carefully with a moist cloth, and the fresh and dry

TABLE VI

COMPARISON OF DIFFERENT VARIETIES OF POTATOES IN LOSS OF DRY WEIGHT FROM MOTHER TUBERS

VARIETY	FRESH WEIGHT OF MOTHER TUBER (GM.)		DRY WEIGHT OF MOTHER TUBER (GM.)		PERCENTAGE DRY WEIGHT LOST
	At planting	After amputation from sprout*	At planting	After amputation from sprout*	
Early Rose.....	20.34	22.56	4.760	1.334	72
King Edward.....	20.33	24.07	3.923	0.956	76
Green Mountain.....	20.37	20.22	5.213	1.333	74
Burbank.....	20.19	19.37	4.656	1.080	77
Russet Rural.....	20.06	25.23	4.339	1.137	74
Peerless Pearl.....	20.66	21.69	4.741	0.696	85
Beauty of Hebron.....	19.60	22.74	4.543	1.286	72
Early Ohio.....	20.03	19.10	4.671	0.742	84
Ehnola.....	20.07	20.57	5.260	0.810	85
McCormick.....	20.85	21.49	3.621	0.859	76

\* Amputation from sprouts after germination had been completed and plants were about 15 inches high.

weights of each again determined (table VI, columns 3 and 5). The right-hand column in table VI shows the percentage of the original dry weight lost by the mother tubers during the period of growth. It is apparent that none of the varieties showed a behavior similar to that of Kruger. All the varieties in the present test lost a large proportion of their dry weight. Early Rose and Beauty of Hebron lost 72 per cent of their original dry weight, and Peerless Pearl and Ehnola lost 85 per cent.

It is interesting to note also in this table the data available on the change in fresh weight of mother tubers during the season. In the previous discussion of the behavior of Irish Cobbler and Bliss Triumph, it was pointed out that only small changes in the weight

of the mother tuber took place, Irish Cobbler losing slightly in weight and Bliss Triumph gaining slightly. Table VI, columns 2 and 3, shows that of the varieties in this test, Green Mountain, Burbank, Peerless Pearl, Early Ohio, Ehnola, and McCormick showed such small changes that it may be doubted whether the amount of change is greater than the errors of measurement. Relatively large increases in fresh weight were shown by King Edward and Russet Rural. SELIBER (17) has emphasized the tendency of the tubers to gain in weight during the season, but in the present experiments the more usual tendency has been for the fresh weight to remain nearly constant. Probably the condition of the seed-piece at the time of planting and the weather conditions during growth are important factors.

### Discussion

It should be emphasized that the results of this series of experiments were obtained with mother tubers 28 gm. in weight. This size was selected because from the literature it appeared that this is about the smallest size that can be depended upon to produce a plant with full vigor. And while in these experiments removal of mother tubers at midseason or later did not markedly reduce the final yield, it is possible that with smaller seed-pieces the influence of the mother tuber would have been exerted during the later periods of growth. Likewise, although a reduction in yield resulted from the removal of mother tubers after germination was complete and leaves were fully formed, it is possible that if large seed-pieces had been used, and if large supplies of stored materials had been available during the early stages of germination, the young plant might have become independent of the mother tuber at an earlier stage. Further work regarding the relation of size to the stage of development at which contact with the seed-piece is no longer critical for growth of the sprout would be desirable.

It may have been noted that the analytical data are presented on the fresh weight and the "per tuber" basis, but not on the dry weight basis. The results of the analyses in these experiments are peculiarly well fitted to emphasize the importance of the selection of the basis on which chemical analyses are calculated, and that erroneous conclusions may result from the use of a basis that is un-

suitable for the purpose. It is clear that in the present case the "per tuber" basis is the best one to show the rate of utilization of the stored substances, since in this way we deal with absolute amounts. The fresh weight basis is nearly as good, since in this particular case the fresh weight of a seed-piece is nearly constant throughout the period of sampling. If the information in table V (showing the absolute amounts of various constituents per mother tuber), and that in table IV (showing the dry weight per mother tuber) is used to calculate the percentage composition on the dry weight basis, some very misleading results are obtained. This method of calculation shows, for example, that at stage 2 the starch percentage of the Irish Cobblers was about 19 per cent lower than at the beginning, when as a matter of fact about 60 per cent of the starch had been lost by that time. Again, this method of calculation shows that little change in amino acid content had taken place between the beginning of the experiment and stage 3; but the mother tuber had lost, in fact, 83 per cent of this constituent. This confusion results from the condition that as the substances are used up the dry weight itself also falls, and the percentage change on this basis depends upon the relative rate of fall of the two changing values.

The observations (table IB, columns 1 and 2) that in midseason it appears possible that the Irish Cobbler plants had become completely independent of the mother tubers, and that amputation may not have influenced the yield unfavorably, are not in accord with the observations of SIKORSKI (18) and SELIBER (17). It must be remembered, however, that they suggest the importance of the mother tuber as an organ for water storage, and this factor would come into play only in periods of drought. In the present experiments there was an abundance of moisture in the soil at all times, and therefore no opportunity was afforded to test their hypothesis. The fact that in the preliminary experiments removal of the mother tuber by cutting across the base of the stem with a knife, or amputating by twisting, induced wilting of the plant even under moist conditions, lends support to the view that the water stored in the mother tuber might become an important factor.

The unfavorable effect upon the yield of Bliss Triumph which was obtained when the mother tubers were allowed to remain in con-

tact with the plant from stage 3 to stage 4 (table IB, columns 3 and 4) may have resulted from toxic materials formed during the rotting of the tubers. The reports of BREAZEAL (3) and COLLISON (5) emphasize the toxic effects of plant tissue during certain stages of decomposition. If toxic substances were formed during the rotting of mother tubers, the conditions for their absorption by the plant would be favorable because of the close connection at the base of the stem between the mother tuber tissue and the sap-conducting vessels.

DE VRIES (6) believed that the mother tuber furnished nutrients to the sprout until germination was complete, after which time its substance was transferred exclusively to the newly developing tubers. LUDWIG (11) adopts this as an argument against large tubers for seed-pieces, on the basis that it is not an economical method, since much of the starch in the large tubers merely passes out of the old into the young tubers, and at the end of six months is harvested a second time. There is evidence against these views in the results of the present experiments. Fig. 2 shows that at stage 2 very few young tubers had begun to form, and that even at stage 3 the new tubers were still small. The analyses (tables II and V, and fig. 5) show that at stage 3 nearly all of the starch and various forms of nitrogen had already been exhausted from the mother tuber; in fact, only 20-25 per cent of the original dry weight still remained, and the subsequent analyses showed that not much more transference from the mother tuber was to take place. Most of the materials destined to leave the mother tuber did so within about forty days from planting, or about twenty days from the date of emergence of sprout. We are not justified in inferring from this that the small amount of substance left at this stage does not exert a considerable influence upon the later growth of the plant, but it suggests that this influence is qualitative, and not due to the quantity of starch or other materials furnished.

This brings up the question as to the nature of the chemicals that pass from the mother tuber to the sprout and strongly influence its growth. Are these materials merely food stuffs, or are they special growth-promoting substances, such as have been postulated by APPLEMAN (1)? The present experiments seem competent to show that materials ordinarily regarded as food stuffs, such as sugar,



amides, amino-acids, etc., are used up rapidly and disappear from the mother tuber, and we must assume that they constitute an important part of the contribution made by the seed-piece. But the experiments are not capable of showing that special substances do not pass also, and that these do not exert an important function in regulating the use of the food stuffs that are translocated. We may regard the situation as analogous to that found by REED (15) in the twigs of lemons and other plants, in which two factors are considered to be operating jointly: first, stored food which (when in the proper condition) can support growth; second, special substances which (in small quantities) influence the utilization of these food materials in growth. REED speaks of them as substances which catalyze the growth process. In any event it is clear that the methods of analysis used in my experiments are not capable of showing the presence of special growth-promoting substances. The use of culture solutions would offer a more favorable method of experimentation; and the results of LUDWIG (11), showing the influence of salt content of the culture solution in modifying the rate of utilization of the stored foods in the tuber, are suggestive of the capacity of small amounts of one substance to control the utilization of a relatively large quantity of other substances in growth.

### Summary

1. A series of experiments, in 1926, 1927, and 1928 is reported, in which the mother tubers were amputated from potato plants at intervals after planting, in order to observe the effect of this removal upon the subsequent growth and yield of the plant, and to note at what stage of development the young plants became independent of the food reserves of the seed-piece.

2. The seed-pieces were planted in the bottoms of pots which were then buried in the soil; when the sprouts had reached certain sizes the pots were removed and inverted, exposing the mother tuber, which was then amputated; the pot was discarded and the well-rooted plant replaced in the soil. The checks consisted of an equal number of plants which were removed from the pots at the same time but which were allowed to retain the mother tubers.

3. The mother tubers were removed at the following periods of

development: stage 1, when the sprouts first emerged from the soil and before leaves had expanded; stage 2, when plants were about 2 inches and had leaves fully expanded; stage 3, when plants were about 10 inches high and young tubers were forming on well-developed stolons; stage 4, when plants had attained approximately maximum height and were in bloom.

4. The effect of amputation upon subsequent growth is shown by photographs, and by tables of the yield. Plants from which the mother tubers were removed at stage 1 gave a yield of tubers only about one-third of that of the checks; removal of the mother tuber at this stage was critical and many plants were not able to survive. The plants from which mother tubers were removed at stage 2 gave a yield which was about 80 per cent of that of the corresponding checks, but the difference in yield was significant statistically, indicating that at this stage of development the young plant has not yet become independent of the stored food in the seed-piece. With respect to the effect of amputation at stage 3, the two varieties used (Irish Cobbler and Bliss Triumph) gave different responses. In the case of Irish Cobbler, plants from which mother tubers were removed showed a reduction in yield per plant, but the odds that this difference was significant were not high, leaving the issue in doubt. In the case of Bliss Triumph, the amputated plants gave a higher yield than the checks, indicating that contact with the mother tuber had been detrimental. It is suggested that this result was due to the rotting of the mother tubers between stages 3 and 4, with the production of toxic substances which were absorbed from the seed-piece at the base of the main stem and unfavorably influenced the subsequent growth. This effect was not observed in the case of Irish Cobbler seed-pieces, since they remained firm between stages 3 and 4. Removal of mother tubers at stage 4 produced no effect upon the yield of Irish Cobbler, but again the yield of Bliss Triumph was slightly greater with mother tubers off than with them on. The difference in this case, however, was not large enough to be conclusive statistically.

5. It appeared possible that the sprout became independent of the mother tuber at stage 3 (about 10 inches high). Most of the storage materials that were destined to leave the mother tuber had

already been used up. Further experiments are required to show conclusively whether contact with the mother tuber at this or later stages is beneficial; and if so whether the advantage results from the transfer of the small amount of food materials still available at that time, or from the water storage relation existing between seed-piece and sprout.

6. These experiments were carried out with mother tubers weighing 28 gm. each. It is possible that with smaller seed-pieces the sprout would have remained dependent on the mother tuber for a longer period, and that with larger pieces amputation could have been carried out at an earlier period without interfering with subsequent development.

7. The amputated mother tissue was subjected to chemical analyses in order to determine the rate at which stored substances left it, and to note any correlation which might exist between the composition at any stage of development and the subsequent behavior. A rapid loss of substance from the mother tuber was noted. By the time the sprout emerged, about one-fourth to one-third of the dry weight was lost; about one-half remained at stage 2; and at stage 3 the mother tubers had been depleted of nearly 80 per cent of the original dry weight. The extent of the depletion varied with different lots, in different years, and with different varieties, but ranged from 70 to 85 per cent. Starch and the various forms of nitrogen (both soluble and insoluble) were steadily used up; but the sugar concentration was maintained at a high level, so that, even though the dry weight had been reduced to a low level the sugar percentage on the fresh weight basis was greater at all subsequent stages than it had been at the time of planting.

8. The analyses showed that different groups of nitrogenous substances, such as insoluble, soluble, ammonia, amide, amino, basic, etc., were removed from the mother tuber at approximately the same rate. There was no evidence of one form being more readily available for growth than other forms.

9. Although between stages 2 and 3 the foliage was active photosynthetically, large demands upon the organic substance of the mother tuber were made during this period. A "sparing" action

upon stored foods in the underground part because of food manufacture in the tops of the plant was not noted.

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## GERMINATION AND KEEPING QUALITY OF PARSNIP SEEDS UNDER VARIOUS CONDITIONS<sup>1</sup>

HILDA C. JOSEPH

(WITH TWO FIGURES)

### Introduction

In several ways parsnip seeds furnish especially favorable material for the study of the effect of storage conditions upon longevity. They are short-lived, and when stored in bags in a laboratory the vitality drops about 20 per cent during two years and 60 per cent during three years of storage; hence the differential effect of storage conditions is quite evident within two years. They withstand extreme drying without injury to their vitality, which makes it possible to study the effect of a wide range of water content. They germinate with reasonable speed over a considerable range of temperature.

The experiments reported in this paper have been conducted for the purpose of finding a method by which parsnip seeds could be stored for several years without losing their vitality. Such a method would be of advantage to seedsmen in enabling them to avoid an annual discard of seeds left over from the previous year. It is also hoped that a method worked out for storage of parsnips might be applicable with modifications to other short-lived seeds of commercial importance, such as cabbage, onion, and a number of the coniferous seeds.

The problems which presented themselves in connection with this study of parsnip seeds were mainly the following: (1) Is the seed material as harvested and sold by the seed grower uniform enough for experimentation, or do the ripe and green seeds of each shipment require separate investigation? (2) What are the optimum temperature requirements for the germination of parsnip seeds, and does the optimum germination temperature remain unchanged when the seeds become older? (3) What influence have moisture content, stor-

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age temperature, humidity of storage chamber, fluctuation of humidity, and aeration upon the keeping quality of the seeds? (4) In which way or ways do the factors mentioned under (3) influence the viability of parsnip seeds?

### Discussion of literature

A number of investigations have been made to determine the influence of various storage conditions upon the keeping quality of seeds. This discussion will be limited, however, to the reports of DUVEL (1), HEINRICH (2), MAQUENNE (3), NAKAJIMA (4), and TILLOTSON (5), which bear directly on the work reported in this paper.

The paper of MAQUENNE is the only one in which experiments with parsnip seeds are reported. MAQUENNE stored parsnips in vacuum for two years at room temperature. During this period the seeds retained their viability completely, while a control lot stored in a paper bag lost all vitality during the same period. Excessive drying, according to MAQUENNE, is the only way of successfully retaining viability in certain seeds, because only when the last traces of water are liberated from the seed under the action of the vacuum is a state of "supermaturation" reached. He defines supermaturation as "a new state of equilibrium established between the enzymes and the substances they condense," and speaks of it as a state of suspended life.

Since MAQUENNE's experiments ran only a little more than two years (1899-1902), it remains to be proved whether he really was able to produce a state of suspended life in his experimental material, or whether the life processes were only retarded enough to leave the final percentage of germination unchanged for a certain time. He does not give data to show whether the germination energy as well as the germination power remained constant. He also fails to show that there is no other way of storing by which vitality could be maintained in short-lived seeds. Since the data given are based on quantities of 2-4 gm. of seeds which were originally not of a very high quality (51 per cent of vital seeds at the beginning of the experiment), it seemed desirable to investigate the keeping quality of a larger number of parsnip seeds of good quality under a variety of storage conditions.

DUVEL (1) worked with a number of vegetable seeds, which he

stored under various climatic conditions in different places of the United States, such as Mobile, Alabama; Wagoner, Indian Territory; and Ann Arbor, Michigan. He determined the effect of various degrees of atmospheric humidity and temperature on the keeping quality of his material, and worked out different methods of packing and sealing seeds as a protection against the influence of high atmospheric humidities. One of his main conclusions is that "as a factor detrimental to vitality, moisture is of far greater importance than temperature."

DUVEL was working with atmospheres of high constant humidity and widely fluctuating humidities without distinguishing between the effects of the two. Later TOUMEY (6) emphasized the detrimental effect of variations in humidity, even when the moisture content reached at different times was not very high, making the following statement:

Even the most resistant species suffer . . . when kept over summer in a loft where the moisture content of the seed is likely to vary with variations in the humidity of the air.

This quotation applies to forest seeds, especially to those of the coniferous type, and is taken from the chapter on seed storage, which contains very good general information as to optimum storage conditions for various tree seeds.

HEINRICH (2) has made significant contributions to our knowledge of the effects of storage conditions upon the vitality of seeds of cereal and forage crops. He determined the rate at which these seeds absorb water from a saturated atmosphere, also the total amount thus absorbed. He also found the "critical moisture content" for seed storage. With moisture content above the critical point there is rapid degeneration of the seeds in storage, while with moisture content below it the vitality is little modified by a considerable period of storage. Discovery of the critical moisture content is one of the very important contributions to our knowledge of seed storage. It is also a fact that has been largely overlooked by later investigators.

TILLOTSON (5) shows the favorable influence of dry air-tight storage upon the retention of vitality by coniferous seeds. His method avoids the injurious effects of fluctuating moisture content. He did not determine the exact moisture content of the seeds, but the



excellent keeping quality shows that they were below HEINRICH'S critical moisture content.

NAKAJIMA'S experiments (4), published in 1927, are interesting because of the methods used for drying the seeds. In addition to drying over desiccating agents, he mixed quicklime or calcium chloride with the seeds, and varied the amount of the drying agent added to suit the amount of desiccation endured by the various seeds, thus avoiding excessive or injurious drying. He considers his method of controlled drying in closed chambers superior to "air-dry" air-tight storage.

### Methods and material

In these experiments one variety of parsnip, commercially called Hollow Crown, was used exclusively. The supply was obtained from two different commercial seed firms in three shipments in the fall of 1924, and from one firm in one shipment in the fall of 1925. The shipments of 1924 (no. 24266, no. 24702, and Hollow Crown Guernsey) were lacking in uniformity, and were therefore separated into ripe and green lots. The shipment of 1925 appeared to be uniform enough to remain unselected. The total number of seeds handled in all experiments approximated 85,000.

All germination tests were carried out in electrically heated ovens, the temperature of which was automatically regulated. The seeds were placed on three thicknesses of filter paper in Petri dishes, in lots of 100 seeds in each dish. The seeds were left unsterilized after it had been determined that the loss, resulting from treatment with various concentrations of uspulun, was more severe than that caused by molding of the seeds. Alternations of temperature were effected by transferring the seeds from one oven to another, with the longer period (5:00 P.M.-9:00 A.M.) at the lower temperature.

The seeds were stored in glass bottles with rubber stoppers. When seeds with reduced moisture content were stored, the stoppers were cemented in the bottles and coated with DeKotinsky cement. Either the germination chambers just mentioned or an ice chest served as storage chamber. In each chamber the seeds remained in darkness except for the short intervals when the doors were opened. The moisture determinations were made on unmacerated seeds in ovens at 103° C., and in a vacuum oven at 72° C.

### Experimental results

Before storage experiments were begun, preliminary studies were made with the different samples of Hollow Crown seeds: (1) to determine the vitality and optimum germination temperature of fresh seeds; (2) to determine the moisture content of air-dry green and ripe seeds; (3) to improve the germination of green seeds by artificial drying.

The vitality of ripe seeds (brown seed coats) proved to be superior to that of seeds not fully ripe (green seed coats). There is no specific optimum germination temperature for ripe or green seeds of recent harvest. As table I shows, they germinate equally well at

TABLE I

GERMINATION OF FRESHLY HARVESTED PARSNIP SEEDS; COMPLETE  
GERMINATION REQUIRED 21 DAYS IN EACH CASE

COLLECTION (1924)	CON- DITION OF SEED	NO. OF SEEDS IN EACH TEST	PERCENTAGE GERMINATION AT					
			15° C.	20° C.	27° C.	32° C.	15°- 20° C.	20°- 32° C.
Hollow Crown no. 24702.	Ripe	2×100	94	88	83	Molded	87	82
Hollow Crown no. 23266.	Ripe	2×100	.....	95	94	Molded	.....	94
Hollow Crown unselected.	Ripe	4×100	.....	95	.....	.....	.....	.....
Hollow Crown no. 24702.	Green	2×100	70	35	65	Molded	.....	73
Hollow Crown no. 24266.	Green	2×100	72	77	68	Molded	70	79

constant or alternating temperatures between 15° and 27° C., while 32° C. is too high for both kinds of seeds.

Simultaneously with the germination tests, moisture determinations were conducted, the results of which are given in table II. They show that in each of the samples tested the green seeds contain a greater amount of hygroscopic moisture than the ripe seeds.

In an attempt to improve the germination of green seeds by lowering their water content to that of ripe seeds, different lots of unripe seeds were dried in various ways (table III). From the data in table III one may draw the following conclusions: (1) Germination of green seeds can be favorably affected by drying in a vacuum oven at 60° C. for four days. This result was confirmed by similar tests with six more series of samples. The average improvement of germination of dried over undried seeds was 16.6 per cent. (2) There is no

direct relationship between water content and germination quality, since all green seeds with improved germination have a very much lower water content than ripe seeds that germinate equally well. (3)

TABLE II

MOISTURE CONTENT OF FRESHLY HARVESTED, AIR-DRY PARSNIP SEEDS;  
NUMBERS REPRESENT AVERAGE OF THREE TESTS

METHOD OF DRYING	NO. OF SAMPLE	AMOUNT OF HYGROSCOPIC MOISTURE IN PERCENTAGE OF DRY WEIGHT	
		Ripe seeds	Green seeds
Vacuum 72° C. ....	24702	6.33	7.46
Vacuum 72° C. ....	24266	6.13	6.50
Access of air 94° C. ....	Guernsey	6.83	6.90

The method of drying is of importance. Drying at room temperature or temperatures between 72° and 85° C. does not affect the germination quality either favorably or unfavorably, while at temperatures above these a marked injury is noticeable.

TABLE III

GERMINATION OF GREEN PARSNIP SEEDS DRIED IN VARIOUS WAYS; NUMBERS  
REPRESENT AVERAGES OF FIVE TESTS WITH 100 SEEDS EACH

DRY TEMPERATURE	ORIGINAL MOISTURE CONTENT	LOSS OF MOISTURE	FINAL MOISTURE CONTENT	PERCENTAGE GERMINATION AT 20° C.
Undried seeds, fresh. ....	7.4	.....	7.4	72.0
Seeds kept at room temperature for 4 months in paper bag. ....	7.4	2.8	4.6	64.2
Fresh seeds dried at 60° C. in vacuum oven for 4 days. ....	7.5	5.6	1.9	83.0
Fresh seeds dried at 72° C. in vacuum oven for 4 days. ....	6.8	7.0	1.8	74.0
Fresh seeds dried at 90° C. with access of air 4 hours. ....	7.4	6.1	1.3	78.0
Fresh seeds dried at 95° C. with access of air 4 hours. ....	6.5	5.6	0.9	23.0
Fresh seeds dried at 103° C. with access of air 2 hours. ....	6.8	4.2	2.6	51.0

This heat injury may be temporary only, provided the seed material is air-dry at the start and well ventilated during the drying. Table IV shows a slight temporary heat injury obtained in five sam-

ples of ripe seeds dried at 87° C. The seeds had completely recovered after one month. On the other hand, a complete destruction of viability occurs even at the optimum drying temperature of 60° C. when the samples are kept in sealed containers while they are heated.

After the percentage of vital seeds in freshly harvested parsnips and the optimum temperature or temperature range for germination had been found, storage experiments were set up in order to determine the influence of humidity, temperature, and ventilation upon the viability of stored seeds, and to find an improved method of storage. The seeds were arranged in the following series: (1) Air-dry seeds of known moisture content were stored in paper bags at

TABLE IV

EFFECT OF HIGH TEMPERATURES ON PARSNIP SEEDS KEPT IN OPEN AND IN CLOSED CONTAINERS; NUMBERS REPRESENT AVERAGE OF FIVE TESTS WITH 100 SEEDS EACH

MATERIAL	DRYING TEMPERATURE	CONDITION OF SAMPLE	GERMINATION AT 20° C.	
			Immediately	One month later
Hollow Crown Guernsey, ripe	87° C. 4½ hours	Weighing bottle uncovered	84.0	96.0
	87° C. 4½ hours	Weighing bottle sealed	None	None
	60° C. 4½ hours	Weighing bottle uncovered	96.7	95.8
	60° C. 4½ hours	Weighing bottle sealed	None	None

room temperature and in an ice chest (5°–7° C.); these seeds were to serve as controls for the following samples. (2) Air-dry seeds of known moisture content were stored in tightly stoppered bottles at 25° and at 5° C. (3) Seeds, the moisture content of which had been reduced to different degrees at various high temperatures, either with access of air or in vacuo, were stored in tightly stoppered bottles at 25° and 5° C. (4) Seeds were treated similarly to those mentioned under (2) and (3), but stored at temperatures between and below those recorded there. This last series is still in progress and will be discussed in a later paper.

When samples of seeds were taken out of the different storage conditions three years later, it was found that the temperature requirements for germination had changed. Green seeds from storage at ice box temperature showed a definite optimum germination at

15° C., instead of growing equally well at constant and alternating temperatures between 15° and 27° C. (table I). Samples of no. 24266, for instance, which had been stored in a paper bag in the ice chest, showed an average germination of 84 per cent at 15° C., 74 per cent at 20° C., and only 58 per cent at 15°-25° C. Green seeds stored at room temperature were all dead, so that all tests remained without results.

Ripe seeds had retained part of their vitality in all storage conditions. Table V shows how the optimum germination temperature had changed in various lots. All samples which had retained a high

TABLE V  
CHANGE IN TEMPERATURE REQUIREMENTS FOR GERMINATION OF RIPE  
PARSNIP SEEDS DURING STORAGE

MATERIAL	WATER CONTENT	METHOD OF STORAGE	OPTIMUM GERMINATION TEMPERATURE (°C.)
Ripe, fresh . . . . .	6.33 and 6.13	.....	15 or 20
Ripe, 3 years old.	Fluctuating 6.33-5.60	Room temperature, paper bag	15-25 alt.
Ripe, 3 years old.	Constant 6.13 and 6.83	Room temperature, sealed bottle	15-25 alt.
Ripe, 3 years old.	Reduced to various degrees, constant	Room temperature, sealed bottle	15
Ripe, 3 years old.	Fluctuating 6.33-9.10	Ice chest, paper bag	15
Ripe, 3 years old.	Constant 6.13 and 6.83	5° C., sealed bottle	15
Ripe, 3 years old.	Reduced to various degrees, constant	5° C., sealed bottle	15

percentage of vitality (as may be seen from tables VI and VII) germinated best at 15° C., while seeds of lower vitality (tables VI and VII) needed alternation of temperature.

Tables VI-VIII show the decrease in germination of the various samples under different storage conditions during the first three years of storage.

The effect of storing seeds in paper bags where they are exposed to variations in atmospheric humidity and temperature is shown in table VI. Ripe seeds retained their viability much better when stored in an ice chest as compared with laboratory storage, in spite of the high humidity in the ice box, which increased the moisture content of the seed considerably. Green seeds lost their vitality faster than ripe seeds under the same cool and humid conditions.

A storage of air-dry seeds in sealed containers under similar

temperature conditions proved very unfavorable at high as well as at cool temperatures (table VII), which shows that fluctuations in

TABLE VI

LOSS OF VITALITY IN VARIOUS COLLECTIONS OF PARSNIP SEEDS DURING STORAGE  
AT ROOM TEMPERATURE AND IN ICE CHEST IN PAPER BAGS

COLLECTION	NO. OF SEEDS USED	MOISTURE CONTENT	LENGTH OF STORAGE PERIOD	STORAGE TEMPERATURE	PERCENTAGE GERMINATION	AT OPTIMUM GERMINATION TEMPERATURE (°C.)	PERCENTAGE LOSS OF VITALITY
Hollow Crown ripe, no. 24202, 1924 . . . . .	$\left\{ \begin{array}{l} 2 \times 100 \\ 3 \times 100 \\ 4 \times 100 \end{array} \right.$	$\left\{ \begin{array}{l} 6.33 \\ 5.60 \\ 5.60 \end{array} \right.$	$\left\{ \begin{array}{l} \text{Fresh} \\ 2 \text{ years} \\ 3 \text{ years} \end{array} \right.$	$\left\{ \begin{array}{l} \text{Room temperature} \\ \text{Room temperature} \end{array} \right.$	$\left\{ \begin{array}{l} 94.0 \\ 72.3 \\ 58.0 \end{array} \right.$	$\left\{ \begin{array}{l} 20 \\ 20 \\ 15-25 \end{array} \right.$	$\left\{ \begin{array}{l} \text{None} \\ 20.6 \\ 39.3 \end{array} \right.$
Hollow Crown ripe, no. 25266, 1924 . . . . .	$\left\{ \begin{array}{l} 2 \times 100 \\ 3 \times 100 \\ 4 \times 100 \end{array} \right.$	$\left\{ \begin{array}{l} 6.13 \\ 9.10 \\ 9.10 \end{array} \right.$	$\left\{ \begin{array}{l} \text{Fresh} \\ 2 \text{ years} \\ 3 \text{ years} \end{array} \right.$	$\left\{ \begin{array}{l} \text{Ice chest} \\ \text{Ice chest} \end{array} \right.$	$\left\{ \begin{array}{l} 95.0 \\ 87.0 \\ 84.0 \end{array} \right.$	$\left\{ \begin{array}{l} 20 \\ 20 \\ 15 \end{array} \right.$	$\left\{ \begin{array}{l} \text{None} \\ 8.5 \\ 11.6 \end{array} \right.$
Hollow Crown green, no. 24266, 1924 . . . . .	$\left\{ \begin{array}{l} 2 \times 100 \\ 3 \times 100 \\ 4 \times 100 \end{array} \right.$	$\left\{ \begin{array}{l} 6.5 \\ 8.8 \\ 8.8 \end{array} \right.$	$\left\{ \begin{array}{l} \text{Fresh} \\ 2 \text{ years} \\ 3 \text{ years} \end{array} \right.$	$\left\{ \begin{array}{l} \text{Ice chest} \\ \text{Ice chest} \end{array} \right.$	$\left\{ \begin{array}{l} 77.0 \\ 54.0 \\ 46.0 \end{array} \right.$	$\left\{ \begin{array}{l} 20 \\ 20 \\ 15 \end{array} \right.$	$\left\{ \begin{array}{l} \text{None} \\ 29.9 \\ 40.4 \end{array} \right.$
Hollow Crown unselected, 1925 . . . . .	$\left\{ \begin{array}{l} 2 \times 100 \\ 3 \times 100 \\ 4 \times 100 \end{array} \right.$	$\left\{ \begin{array}{l} 6.8 \\ 8.6 \\ 8.6 \end{array} \right.$	$\left\{ \begin{array}{l} \text{Fresh} \\ 1 \text{ year} \\ 2 \text{ years} \end{array} \right.$	$\left\{ \begin{array}{l} \text{Ice chest} \\ \text{Ice chest} \end{array} \right.$	$\left\{ \begin{array}{l} 94.0 \\ 90.3 \\ 78.0 \end{array} \right.$	$\left\{ \begin{array}{l} 20 \\ 20 \\ 15 \end{array} \right.$	$\left\{ \begin{array}{l} \text{None} \\ 4.0 \\ 17.1 \end{array} \right.$

TABLE VII

KEEPING QUALITY OF PARSNIP SEEDS WITH ORIGINAL WATER CONTENT STORED  
IN TIGHTLY STOPPERED BOTTLES AT VARIOUS TEMPERATURES;  
4 X 100 SEEDS USED IN EACH GERMINATION TEST

MATERIAL DESCRIPTION	LENGTH OF STORAGE PERIOD	STORAGE TEMPERATURE (°C.)	PERCENTAGE GERMINATION	AT OPTIMUM GERMINATION TEMPERATURE (°C.)	PERCENTAGE LOSS OF VITALITY (°C.)
Hollow Crown ripe, no. 24702, 1924 moisture content 6.33% . . . . .	$\left\{ \begin{array}{l} \text{Fresh} \\ 2 \text{ years} \\ 3 \text{ years} \end{array} \right.$	$\left\{ \begin{array}{l} \text{ } \\ 25 \\ 25 \end{array} \right.$	$\left\{ \begin{array}{l} 88.0 \\ 22.0 \\ \text{None} \end{array} \right.$	$\left\{ \begin{array}{l} 20 \\ 20 \\ \text{ } \end{array} \right.$	$\left\{ \begin{array}{l} \text{ } \\ 75 \\ 100 \end{array} \right.$
Hollow Crown ripe, no. 24266, 1924 moisture content 6.13% . . . . .	$\left\{ \begin{array}{l} \text{Fresh} \\ 6 \text{ months} \\ 2 \text{ years} \\ 3 \text{ years} \\ 3 \text{ years} \end{array} \right.$	$\left\{ \begin{array}{l} \text{ } \\ 25 \\ 25 \\ 25 \\ 5 \end{array} \right.$	$\left\{ \begin{array}{l} 95.0 \\ 97.0 \\ 60.7 \\ 48.0 \\ 54.0 \end{array} \right.$	$\left\{ \begin{array}{l} 20 \\ 20 \\ 20 \\ 15-32 \\ 15 \end{array} \right.$	$\left\{ \begin{array}{l} \text{ } \\ \text{None} \\ 37.5 \\ 50.6 \\ 47.4 \end{array} \right.$
Hollow Crown green, no. 24702, 1924 moisture content 7.46% . . . . .	$\left\{ \begin{array}{l} \text{Fresh} \\ 2 \text{ years} \\ 3 \text{ years} \\ 3 \text{ years} \end{array} \right.$	$\left\{ \begin{array}{l} \text{ } \\ 25 \\ 25 \\ 5 \end{array} \right.$	$\left\{ \begin{array}{l} 35.0 \\ 1.7 \\ \text{None} \\ \text{None} \end{array} \right.$	$\left\{ \begin{array}{l} 20 \\ \text{ } \\ \text{ } \\ \text{ } \end{array} \right.$	$\left\{ \begin{array}{l} \text{ } \\ 95.2 \\ 100 \\ 100 \end{array} \right.$
Hollow Crown ripe, Guernsey, 1924 moisture content 6.83% . . . . .	$\left\{ \begin{array}{l} \text{Fresh} \\ 2 \text{ years} \\ 3 \text{ years} \\ 3 \text{ years} \end{array} \right.$	$\left\{ \begin{array}{l} \text{ } \\ 25 \\ 25 \\ 5 \end{array} \right.$	$\left\{ \begin{array}{l} 95.0 \\ 75.0 \\ 10.0 \\ 12.0 \end{array} \right.$	$\left\{ \begin{array}{l} 20 \\ 20 \\ 15-25 \\ 15 \end{array} \right.$	$\left\{ \begin{array}{l} \text{ } \\ 20 \\ 85 \\ 83 \end{array} \right.$

humidity and constant atmospheric moisture are less injurious to seed vitality than lack of ventilation for seeds of ordinary moisture content. There is an indication that the critical moisture content found by HEINRICH (2) for his seeds is somewhat below 6.13 per cent

TABLE VIII

KEEPING QUALITY OF SEEDS WITH REDUCED MOISTURE CONTENT STORED IN  
TIGHTLY STOPPERED BOTTLES AT VARIOUS TEMPERATURES;  $4 \times 100$   
SEEDS USED FOR EACH GERMINATION TEST

DESCRIPTION OF MATERIAL	TREATMENT	MOISTURE CONTENT	LENGTH OF STORAGE PERIOD	STORAGE TEMPERATURE (°C.)	PERCENTAGE GERMINATION	OPTIMUM GERMINATION TEMPERATURE (°C.)	PERCENTAGE LOSS OF VITALITY
Hollow Crown no. 24266, 1924 green	Dried 4 hours 90° C.	1.27	None	.....	83.0	20	None
		1.27	2 years	25	67.5	20	18.7
		1.27	3 years	25	59.0	15	29
		0.60	3 years	5	70.0	15	15.7
	Dried 24 hours 72° C. in vacuo	1.7	None	.....	61.0	20	None
		1.7	2 years	25	76.0	20	Improved
		1.7	3 years	25	75.0	15	Improved
		1.25	None	.....	60.0	20	None
	Dried 1 hour 92° C.	1.25	2 years	25	51.0	20	15
		1.25	3 years	25	39.5	15	34.2
1.60		3 years	5	47.5	15	20.8	
Hollow Crown ripe, Guernsey 1924.....		Dried 2 hours 92° C.	0.40	None	.....	80.0	20
	0.40		2 years	25	69.0	20	13.8
	0.40		3 years	25	50.0	15	37.5
	0.60		3 years	5	77.0	15	3.8
	Dried 4 hours 92° C.	0.40	None	.....	84.0	20	None
		0.40	2 years	25	75.5	20	10
		0.40	3 years	25	64.0	15	23.8
	Dried 6 hours 92° C.	0.50	None	.....	71.5	20	None
		0.50	2 years	25	73.0	20	None
		0.50	3 years	25	69.0	15	None
0.10		3 years	5	71.0	15	None	

in ripe parsnip seeds, since seeds with this moisture content keep almost as well as artificially dried seeds of a much lower water content. The keeping quality decreases rapidly, however, with an increase of hygroscopic moisture above 6.13 per cent. Again, green seeds show a greater sensitivity than ripe seeds.

The data in table VIII show that the unfavorable influence of lack of ventilation is avoided if the water content of the seeds is reduced by artificial drying. For green seeds the last method of

storage seems to be the only favorable one. It excludes fluctuating and high atmospheric humidities, to which green seeds have been shown previously to be very sensitive.

The fact that ripe seeds also retain their viability better when

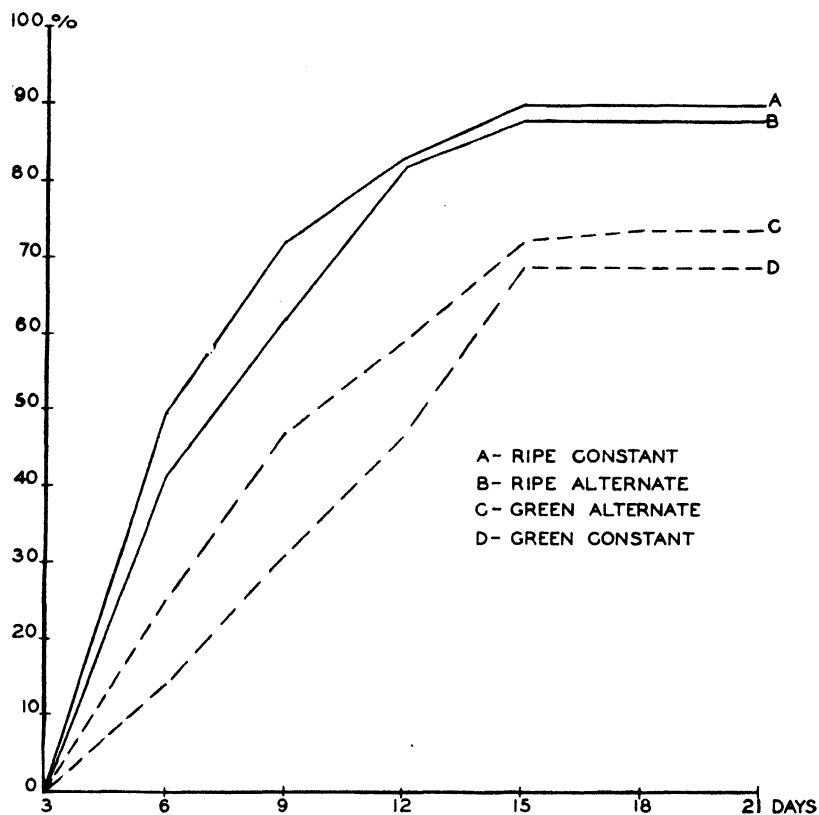


FIG. 1.—Rate of germination in ripe and green parsnip seeds at constant and alternating temperatures immediately after harvest.

stored artificially dried under exclusion of air is shown by a comparison of data in tables VII and VIII.

In addition to the loss in percentage of germination and a shifting in the optimum germination temperature, a slower rate of germination can be noted in stored seeds as compared with freshly harvested materials (figs. 1 and 2).

The germination rate in fresh seeds is highest during the first nine



days and decreases slightly during the following days. Germination is complete after the first fifteen days. The curves for green and ripe seeds are similar in shape, and those for alternating and for constant

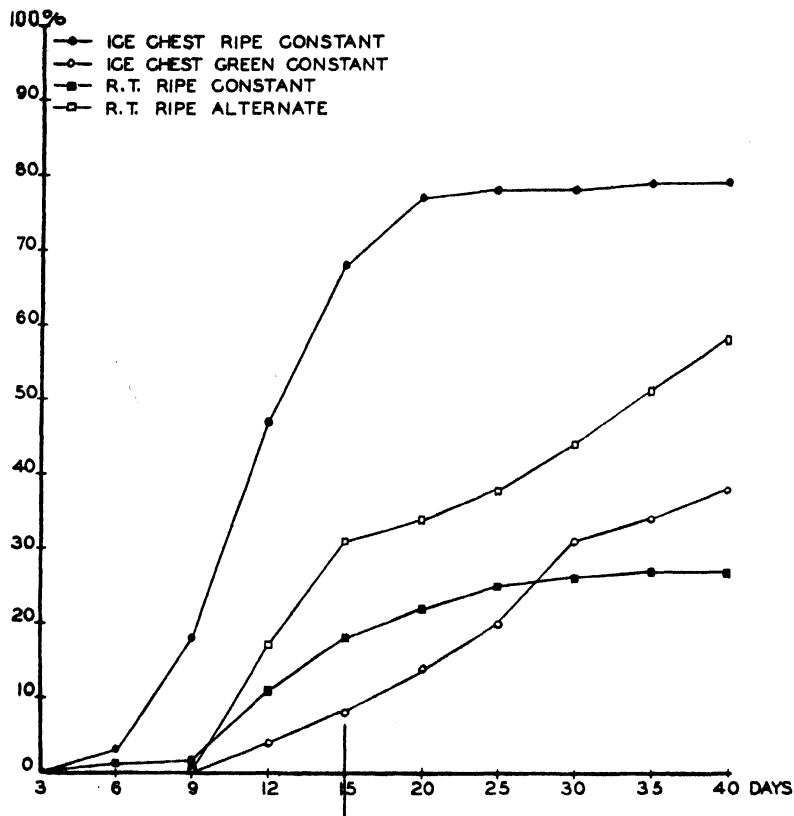


FIG. 2.—Rate of germination in parsnip seeds after three years of storage at room temperature and in ice chest with access of air.

temperatures run close together in both kinds of seeds. After three years of storage, however, curves plotted from similar tests have a very different form. For the first six to nine days the rate of germination is very low, but rises considerably during the following ten days. In seeds of well preserved vitality, such as ripe seeds stored in the ice chest, the optimum percentage of germination is almost reached at the end of this second period, which means that complete germina-

tion is reached after approximately twenty days, five days later than in freshly harvested seeds. In seeds of lower vitality, however, such as ripe seeds stored at room temperature or green seeds, germination proceeds slowly for more than one month, the highest percentage being reached only after forty days. It is also noticeable that the curves obtained from tests with stored seeds lack in regularity as compared with those obtained from experiments with new seeds. This may be due partly to infection by molds, to which stored seeds seem more susceptible than new seeds, but it may also be due to irregularity in absorption of water by stored seeds. In this respect the very low percentage of germination obtained in all stored seeds during the first six days is of special interest. Even seeds of high vitality, such as ripe seeds stored in the ice box, show this decrease in the rate of germination during the first few days. Later the factor which causes this retardation apparently is overcome, and the rest of the curve has the same shape as that for fresh seeds. In seeds of lower vitality, however, the rate remains low throughout the germination period.

### Discussion

A comparison of the experimental results reported here with those obtained by MAQUENNE (3) shows that there are several ways of retaining vitality in parsnip seeds besides the one used by MAQUENNE. The writer was able to obtain almost equally good germination (94 and 90.3 per cent) in two consecutive years with parsnip samples of high quality stored under conditions which permit the continuation of metabolic processes (table VI). The writer has also been able to improve the vitality in green seeds during a period of three years (61-75 per cent germination) by storage at reduced moisture content after drying with artificial heat (table VIII). These storage conditions also did not exclude all life processes. It appears, therefore, that parsnip seeds can retain their vitality under certain storage conditions without being transferred to a state of "super-maturation" for at least as long a period as has been used by MAQUENNE in his experiments.

In agreement with the results of DUVEL (1), it was found that temperature and humidity are the main factors to be considered in parsnip storage; that they are closely dependent upon each other in

their effect on seed viability; and that humidity is the more important of the two.

These facts should be taken into account by nurserymen and foresters when they have to store short-lived seed material. At the present time seed growers suffer great losses from an annual discard of seeds which they are unable to sell after the first winter of storage, and foresters find it difficult to hold over a part of their harvest of short-lived fir and pine seeds from one year to another. Since most firs and pines are biennial or periodic bearers, and good harvests are therefore obtained only every second year or at longer periods, it is hard to find enough seed material of good quality for yearly plantings.

With a knowledge of the exact amount of moisture which may be retained in a seed at available storage temperatures without serious injury to seed vitality, it will be much easier for seed growers to store seeds with minimum loss of vitality for several years. For parsnip seeds this "critical moisture content" for ordinary room temperatures has been determined to lie below 6.13 per cent. With a hygroscopic moisture of more than 6.13 per cent the keeping quality of the seeds decreases markedly.

The main symptoms of devitalization, aside from a gradual loss in percentage of germination, are retardation in rate of germination and need of temperature alternations for germination. DUVEL, who has also noticed a decreasing rate of germination in his dry storage material, concludes that the seed coat must become impermeable to water and retard the absorption of moisture necessary to germination. This is not the case in parsnip, however, because the same retardation in germination rate can be observed when the dried seed coats are broken before the seeds are placed in the germination chamber.

Various other possible causes for slow devitalization in dry storage have been suggested by other workers, such as a colloidal rearrangement of substances in the embryo or a gradual denaturing of protoplasmic cell contents, but the experimental proof for these theories has not yet been obtained.

### Summary

1. In germination tests conducted shortly after harvest, well-ripened brown seeds give better germination than green seeds.

2. The germination of green seeds can be improved through artificial drying at 60° C. in vacuo for four days.

3. There is no definite optimum germination temperature for freshly harvested seeds, constant and alternating temperatures between 20° and 27° C. being equally favorable.

4. With increasing age, parsnip seeds stored under unfavorable conditions require a temperature alternation of 15°–25° C. for germination, while seeds stored under conditions favorable to the retention of vitality germinate best at 15° C.

5. Stored in paper bags at room temperature, ripe parsnip seeds lose their vitality at a rate of 20 per cent during the first two years and approximately 60 per cent during three years of storage, when the moisture content of air-dry seeds is 6.33 per cent in the beginning and 5.6 per cent at the end of the storage period.

6. There are various ways by which the keeping quality of seeds may be improved. At a temperature of 5°–7° C. in an ice box the seeds keep for a considerably longer period, although their moisture content increases in the moist atmosphere of an ice box to more than 1.5 times that of seeds stored at room temperature (9.1 as compared with 5.6 per cent).

7. If the seeds are dried carefully and thoroughly, either for twenty-four hours in vacuo at 72° C. or for four to six hours at 90° C., to a moisture content of 0.40 to 1.7 per cent, their viability remains high even if they are stored at room temperature. To insure a continuous low moisture content, seeds treated in this way have to be kept in air-tight storage. The "critical moisture content" of parsnip seeds lies somewhat below 6.13 per cent.

8. Seeds which are not artificially dried cannot be stored under exclusion of air without losing their vitality very rapidly. Although a low storage temperature retards the death rate of unaerated moist seeds, it does not remove the injurious effect of lack of ventilation.

9. In advising practical storage methods for parsnip seeds, three methods of storing are suggested: (1) Storage at ice box temperature (approximately 5° C.) with frequent stirring of the seeds to secure

good ventilation; with this way of handling, even a very high atmospheric humidity is of little importance to the keeping quality of the seed. (2) When a higher storage temperature has to be used, a thorough drying of the seeds ( $90^{\circ}$  C. for four to six hours) and a subsequent air-tight storage in sealed containers insure good keeping quality. (3) Optimum keeping quality should be obtained where artificially dried seeds are stored air-tight at low temperatures.

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## CHEMICAL CHANGES INDUCED IN POTATO TUBERS BY TREATMENTS THAT BREAK THE REST PERIOD<sup>1</sup>

F. E. DENNY

### INTRODUCTION

In previous articles (2, 3), it was shown that treatments with sodium thiocyanate and ethylene chlorhydiin shortened the dormant period of freshly harvested tubers of *Solanum tuberosum* L. and induced early sprouting, growth of buds becoming distinctly visible in about five to seven days. The experiments here reported upon were undertaken to determine what chemical changes accompanied this resumption of growth in dormant buds, and to note how soon after the treatments these chemical changes occurred.

Samples of treated and check tissue were taken at intervals after treatment, starting as early as 48 hours, and extending up to six days, at which time sprouts 1-2 mm. in length were often obtained. Sampling was not continued beyond this stage since the object was to study only the early stages of growth following the breaking of dormancy.

The most noteworthy changes observed were (1) an increase in sucrose in the treated tissue, and (2) an increase in solids soluble in 50-percent alcohol. These changes were found to occur within at least 48 hours after the end of the period of treatment. Sampling would need to begin at an earlier period than was adopted in this series of experiments in order to ascertain how much sooner after treatment these changes started.

Since a previous experiment (4) with lilac (*Syringa vulgaris*, L.) had shown that dormancy was localized narrowly in the buds, samples of the eye-tissue in the potatoes were collected separately from the rest of the seed-piece, and the analyses were compared with those obtained from the tissue-not-at-eye. It was found that these changes in sucrose and soluble solids occurred in both types of tissue, but that the changes were greater in the tissue-at-eye than in tissue-not-at-eye.

### METHODS

#### Treatment of Tissue and Collection of Samples

The experiments were arranged to furnish comparable samples of treated and check tissue at various intervals after treatment. The tubers

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

were cut into pieces approximately 28 grams in weight, each seed-piece having one eye only. For each pair of samples to be taken after treatment about 700 seed-pieces representing about 175 tubers were used; the number of tubers involved in any single experiment, therefore, ranged from about 175 to 525. The seed-pieces were divided into two lots, one lot being treated with the chemical (either by the dip-method using ethylene chlorhydrin, or by the soak-method using sodium thiocyanate), and the other lot being the check (dipped or soaked in water instead of chemical). The treated potatoes were then planted in the soil in flats and allowed to stand until a subsequent period at which time a sample of about 350 pieces each of treated and check lots were removed, and tissue taken for analysis. It will be noted in tables 1 and 2 that sampling began 48 to 72 hours after treatment and extended up to 144 hours; the number of consecutive samples obtained varied with the different lots; usually only one was obtained, but in some cases two were taken, and from one lot three successive samples (two, four, and six days after treatment) were available.

After the seed-pieces were removed from the soil they were wiped with a moist rag. In obtaining samples of eye-tissue a chip of tissue just above the sunken eye was cut off and discarded, leaving a smooth, level surface with the eye in the center; the eye was then picked out with a sharp knife, about 0.1 gram of tissue being removed in this process; two samples (25 and 10 grams each) were obtained at each sampling period. In removing the eye tissue a certain amount of the surrounding tissue was included, but care was taken to include approximately the same amount of this adjacent tissue in different samples, success in this respect being attained by weighing a number of the eye-pieces occasionally, and noting whether the average weight checked closely to 0.1 gram.

After the eyes were removed the rest of the seed-piece was sampled for tissue-not-at-eye as follows: the skin and the suberized surfaces were trimmed off by the removal of a thin layer, and the tissue was then chopped finely in a wooden bowl. Two samples for moisture were weighed out and two samples (100 grams each) were taken for the subsequent analyses.

### **Analytical Methods**

The weighed samples of tissue were dropped into boiling alcohol, the amount of alcohol being adjusted to give a final concentration of about 70-percent alcohol, taking into account the moisture in the tissue. The tissue was stored for subsequent analyses in this concentration of alcohol, but when the analyses were begun, water was added to make the alcoholic concentration 50 percent by volume. The procedure in extracting the tissue and in carrying out analyses of the soluble and insoluble fractions was the same as has been described in a previous paper (5), except with respect to the determination of starch. In the present experiments starch was estimated by the Walton and Coe (7) method which eliminates interfering

polysaccharides, and which therefore gives a more dependable value for the starch content. The moisture percentage that was obtained from samples of the tissue-not-at-eye was used in the calculations. At the time of obtaining the tissue-at-eye samples, tissue could not be spared for separate determinations of moisture. Subsequently it was found that eight of the tissue-at-eye samples in alcohol could be used for making a comparison of the moisture-at-eye with that in the balance of the seed-piece by evaporating down to dryness the sample of eye-tissue stored in alcohol, and weighing the residue. In six of the eight cases the eye-tissue showed a lower water content, the decrement averaging 0.6 percent of the moisture content found in the corresponding not-at-eye tissue.

### Varieties and Source of Tubers

Three varieties were included in the tests: Bliss Triumph, Irish Cobbler, and Garnet Chili. The tubers for lots nos. 1 and 4 (see column 1 in each table) were obtained from Bermuda through the courtesy of the Bermuda Department of Agriculture; those for lots nos. 2 and 3 were supplied by the Everglades Experiment Station of the University of Florida; those for lots 6 and 8 were furnished by the Office of Horticulture of the United States Department of Agriculture; and the tubers for the other lots were obtained from the Institute gardens.

Seed-pieces representing the treated and check lots in each experiment were planted in soil in boxes for later observations regarding the effects of the treatments upon the rate of germination. In all cases the treated tubers germinated sooner than the checks, the difference being less pronounced, however, for lots no. 1 and 11. Photographs of the treated and check lots were obtained at later stages of growth but these are not published for the reason that they are not essentially different from photographs published in previous articles (2, 3).

### RESULTS

The results of the chemical analyses are given in tables 1, 2, and 4, the data being given as percentages of the fresh weight of the tissue. Table 1 shows the results with the tissue taken at only the eyes of the potatoes. The most noteworthy difference in composition between treated and check lots is shown by the figures for sucrose in column 9. There are 13 samples available for comparison and in each case the sucrose of the treated lot is higher than in the corresponding check. This is true even of the samples taken only 48 hours after the end of the treatment.

The sucrose determinations were carried out mainly by the use of hydrochloric acid as the inverting agent (see 1, p. 95), but in some cases the results obtained in this way were checked by the use of the enzyme invertase. Since the results by the two methods were in agreement it seems likely that the increase in reducing power was due to the presence of



TABLE 1. *Chemical Composition of Treated and Check Tissue. Samples of Potato Tissue Taken at Eye Only \**

Lot No.	Variety **	Chemical Treat- ment Used †	Days After Treat- ment	Percentage Composition on the Basis of the Fresh Weight of the Tissue											
				Soluble Solids †† %		Reducing Sugar %		Sucrose %		Starch %		Insoluble § Nitrogen %		Soluble §§ Nitrogen %	
				Check	Treated	Check	Treated	Check	Treated	Check	Treated	Check	Treated	Check	Treated
1	Bliss	Chlorhydrin	5	3.06	3.88	0.358	0.358	0.412	0.888	13.61	11.85	0.349	0.328	0.119	0.138
2	Cobbler	"	5	3.15	4.18	0.040	0.145	0.394	1.116	13.33	14.33	0.361	0.301	0.138	0.178
3	"	"	2	3.14	4.00	0.280	0.062	0.287	0.524	10.28	9.40	0.275	0.293	0.136	0.156
4	Garnet	"	5	3.60	3.62	0.454	0.241	0.431	0.602	12.85	11.86	0.266	0.256	0.112	0.128
5	Bliss	"	2	2.95	3.15	0.194	0.247	0.207	0.805	8.88	7.91	0.225	0.207	0.100	0.080
6	"	"	4	2.85	3.60	0.194	0.235	0.252	0.693	9.52	9.39	0.206	0.151	0.130	0.130
7	Cobbler	NaSCN	3	2.49	3.12	0.203	0.159	0.176	0.682	8.39	8.26	0.250	0.239	0.087	0.110
			2	3.10	3.30	0.105	0.171	0.229	0.379	10.31	9.66	0.223	0.228	0.150	0.162
8	Bliss	Chlorhydrin	5	3.12	3.89	0.171	0.320	0.257	0.953	10.62	10.22	0.214	0.250	0.165	0.154
9	"	NaSCN	3	2.32	3.20	0.209	0.176	0.170	0.586	8.63	8.44	0.257	0.256	0.080	0.113
			2	3.36	3.81	0.247	0.367	0.252	0.570	7.81	5.67	0.256	0.258	0.110	0.120
10	"	Chlorhydrin	4	3.20	3.77	0.218	0.547	0.183	0.799	8.12	6.51	0.232	0.242	0.122	0.110
			3	2.67	3.36	0.241	0.259	0.202	0.375	8.49	7.75	0.230	0.238	0.104	0.123

\* Sample of tissue-at-eye obtained by removing about one-tenth gram of tissue at the eye of the seed-piece.

† These names are shortened designations for Bliss Triumph, Irish Cobbler, and Garnet Chili.

†† Refers to solids soluble in 50% alcohol by volume.

§ Nitrogen (N) insoluble in 50% alcohol by volume.

§§ Nitrogen (N) soluble in 50% alcohol by volume.

sucrose in the original solution. However, the possibility of the presence of other substances, such as glucosides, from which increased reducing power could result during the procedure used, has not been excluded with certainty in these experiments.

The solids soluble in 50-percent alcohol also show increases in the treated lots in all cases except in lot no. 4 (see column 5, table 1). The reducing-sugar data do not show consistent differences between treated and check samples (see column 7, table 1); in eight out of 13 comparable pairs the reducing sugar was higher in the treated lots, but in four of the cases it was lower. The reducing sugar results were too variable in these tests to justify a conclusion as to the behavior of this constituent during the period shortly after the chemical treatments. A decrease in starch in the treated tissue was observed in 12 out of the 13 cases (see column 11, table 1). The change in starch is small, however, and the behavior of lot 2 in showing an apparent increase in starch in the treated lot indicates that further study should be made. In particular other polysaccharides besides starch may be important and a quantitative determination of each group should be made.

The analyses of the nitrogenous substances soluble and insoluble in 50-percent alcohol are shown in table 1, columns 13 to 16. The data are not consistent enough to permit a conclusion as to the effect of the chemical treatment upon the nitrogenous materials. In most cases the soluble nitrogen was found to be greater in the treated lots than in their corresponding checks. But these increases in soluble nitrogen have not been definitely correlated with decreases in the insoluble nitrogen. In view of these facts it seems undesirable to attempt an interpretation of the data on the nitrogen-containing compounds at the present time. The present experiments indicate that the changes in carbohydrates have been greater than those in the nitrogenous groups.

The analyses of the samples of tissue-not-at-eye are shown in table 2. The lot numbers used in table 2 refer in each case to the corresponding lot numbers in table 1, and therefore refer to the same sample of tubers. In addition five other analyses were available for this type of tissue, making 18 different samples (from 13 different lots of tubers) that are available for the comparison of treated and check tissue.

The differences observed for tissue-not-at-eye (table 2) are qualitatively the same as those for tissue-at-eye (table 1). Here again the outstanding changes are shown by the sucrose and soluble solids data (see table 2, columns 9 and 5). In each case the cane sugar percentage was greater in the treated tissue than in the check, and although the gain in soluble solids is not so great as that for the cane sugar the data are consistent and convincing. The reducing sugar values do not show consistent differences between treated and check lots, and the situation with respect to nitrogenous substances is about the same for the tissue not-at-eye as for tissue-at-eye.

TABLE 2. *Chemical Composition of Treated and Check Tissue. Samples Taken from Tissue-not-at-Eye of Potato \**

Lot No.	Variety	Chemical Treatment Used	Days After Treatment	Percentage Composition on the Basis of the Fresh Weight of the Tissue													
				Soluble Solids %		Reducing Sugar %		Sucrose %		Starch %		Insoluble Nitrogen %		Soluble Nitrogen %		Moisture %	
				Check	Treated	Check	Treated	Check	Treated	Check	Treated	Check	Treated	Check	Treated	Check	Treated
1	Bliss	Chlorhydrin	5	3.06	3.32	0.341	0.173	0.357	0.577	16.31	15.67	0.167	0.138	0.162	0.183	76.3	76.5
2	Cobbler	"	5	3.10	3.40	0.234	0.107	0.378	0.573	12.42	13.01	0.215	0.178	0.210	0.224	78.0	77.8
3	"	"	2	2.37	2.67	0.335	0.229	0.290	0.475	12.04	11.54	0.186	0.179	0.175	0.186	80.3	80.8
4	Garnet	"	5	2.87	3.00	0.441	0.347	0.557	0.591	13.14	13.58	0.149	0.129	0.157	0.160	79.3	79.3
5	Bliss	"	2	2.32	2.42	0.047	0.088	0.369	0.565	9.59	8.89	0.170	0.180	0.144	0.105	83.7	84.2
6	"	"	4	2.14	3.02	0.059	0.194	0.256	0.497	9.70	9.47	0.182	0.141	0.140	0.147	84.1	84.0
7	Cobbler	NaSCN	3	1.86	2.16	0.177	0.106	0.179	0.474	10.29	10.63	0.189	0.179	0.102	0.121	83.7	83.2
8	"	"	5	2.57	2.65	0.100	0.144	0.218	0.350	11.37	10.66	0.157	0.165	0.148	0.135	81.4	82.1
9	Bliss	Chlorhydrin	3	1.67	2.36	0.167	0.268	0.156	0.606	10.80	10.05	0.161	0.165	0.150	0.133	82.0	81.0
10	"	NaSCN	2	2.89	3.09	0.148	0.151	0.312	0.572	8.19	8.16	0.160	0.165	0.150	0.162	84.3	83.4
11†	"	"	4	2.96	3.53	0.141	0.305	0.363	0.874	7.45	6.89	0.155	0.151	0.165	0.154	84.9	85.2
12†	"	Chlorhydrin	3	2.39	2.42	0.315	0.159	0.271	0.447	8.95	8.93	0.180	0.186	0.135	0.132	84.7	84.3
13	Cobbler	"	6	2.22	2.67	0.265	0.337	0.361	0.322	11.18	10.60	0.125	0.122	0.140	0.148	84.1	84.3
	"	"	6	2.29	2.56	0.029	0.035	0.130	0.218	11.24	12.07	0.161	0.141	0.170	0.191	81.2	81.2
	"	"	2	2.41	3.34	0.023	0.023	0.218	0.269	11.63	10.99	0.193	0.156	0.179	0.178	81.5	81.8
	"	"	4	2.50	3.56	0.021	0.026	0.204	0.322	11.43	10.48	0.178	0.134	0.184	0.186	82.2	83.1
	"	"	6	2.22	2.74	0.021	0.023	0.182	0.235	12.86	11.02	0.171	0.146	0.156	0.195	81.5	83.0

Note: Lot numbers, varieties, treatments, and other details correspond to those in table 1.

\* Tissue-not-at-eye obtained by peeling the seed-piece after removal of eye-tissue, and taking the sample after mincing the tissue.

† Includes eye-tissue also.

The differences in starch between the treated lots and their corresponding checks are not fully consistent in the various lots (see table 2, column 11), eight samples showing decreases in starch and three showing increases. However, the average percentage decrease is significant statistically by Student's (6) method.

### Percentage Change of Sucrose, Soluble Solids, and Starch

In order to show the amount of change observed with respect to sucrose, soluble solids, and starch, the increase or decrease of the treated lots was calculated as a percentage of the corresponding check lot, and these data are shown in table 3. The results with the tissue-at-eye are shown in columns 3, 4, and 5, and those with the tissue-not-at-eye in columns 6, 7 and 8.

TABLE 3. *Percentage Change in Chemical Constituents at Intervals After Treatment*

Lot No.	Days After Treatment	Difference Between Treated and Check Lots Expressed as Percentage of the Check. (+) = Increase in Treated Lot; (-) = Decrease in Treated Lot					
		Samples of Tissue Taken at Eye Only *			Samples Taken from Tissue-not-at-eye †		
		Soluble § Solids %	Sucrose %	Starch %	Soluble § Solids %	Sucrose %	Starch %
1	5	+27	+115	-13	+ 9	+ 61	-4
2	5	+23	+183	+ 8	+10	+ 51	+5
3	2	+27	+ 83	- 9	+13	+ 64	-4
4	5	0	+ 40	- 8	+ 5	+ 6	+3
5	2	+ 7	+289	-10	+ 4	+ 53	-7
	4	+26	+175	- 1	+41	+ 94	-2
6	3	+25	+288	- 2	+16	+164	+3
7	2	+ 6	+ 66	- 6	+ 3	+ 60	-6
	5	+25	+271	- 4	+17	+288	-7
8	3	+38	+244	- 2	+41	+120	-3
9	2	+13	+126	-27	+ 7	+ 83	0
	4	+18	+337	-20	+19	+140	-8
10	3	+26	+ 86	- 9	+ 1	+ 65	0
Average		+20	+177	- 8.0	+14	+ 96	-2.3

*Note:* Lot numbers, varieties, and chemical treatments correspond to those in tables 1 and 2.

\* Tissue-at-eye obtained by removing approximately one-tenth gram of tissue at eye of seed-piece (see text).

† Tissue-not-at-eye obtained by peeling the seed-piece after removal of eye-tissue and taking the sample after mincing the tissue.

§ Solids soluble in 50-percent alcohol (by volume).

Table 3 (columns 4 and 7) shows that the percentage increase in sucrose is consistent with respect to the various samples, and surprisingly high in value. To a somewhat less extent this difference was true also with respect to the solids soluble in 50-percent alcohol (see table 3, columns 3 and 6), all comparable pairs but one showing a greater amount of soluble solids in the treated lots. The differences in starch were not found to be so great

numerically nor so consistent in the different lots, but the observed average decreases in starch in the treated lots are statistically significant.

Table 3 also permits a comparison of the differences between tissue-at-eye and tissue-not-at-eye with respect to the amount of change in chemical composition. This comparison is interesting in view of the results previously reported for lilac (4) in which it was shown that the dormancy was localized narrowly in the bud. If this is true of potatoes also, we should expect the changes in composition to be greater in the tissue-at-eye than in tissue-not-at-eye. The sucrose changes in table 3, columns 4 and 7, show that the cane sugar in the eye-tissue of the treated lots was 177 percent higher than in the corresponding checks, while the increase in tissue-not-at-eye was 96 percent. By Student's method of estimating the significance of differences it can be shown that the odds are about 400 to 1 that the sucrose change was greater at the eyes than at the rest of the seed-piece. The difference between the gains at-eye as compared with not-at-eye is less for the solids soluble in 50-percent alcohol (table 3, columns 3 and 6) but, using the same method of statistical comparison, odds of 27 to 1 are obtained that the increase is greater in the eye-tissue. In a similar manner the starch analyses (see table 3, columns 5 and 8) show that the samples of the tissue-at-eye in the treated lots were 8.0 percent lower in starch than their checks, while for tissue-not-at-eye the treated lots averaged 2.3 percent lower in starch; calculations by Student's method show odds of 60 to 1 that the decrease in starch was greater in the tissue-at-eye than in tissue-not-at-eye.

These results corroborate the view that the initial changes take place at least in the vicinity of the bud, if not in the growing-point itself.

### **Effect of Treatment Upon Different Forms of Soluble Nitrogen**

In addition to the determinations of the soluble and insoluble forms of nitrogen shown in tables 1 and 2, some experiments on the forms of nitrogen present in the soluble portions were carried out. Aliquots of the 50-percent alcoholic extract were used and the results are given in table 4. It will be seen that no consistent differences between treated and check lots were found, the agreement between the two being in most cases good. There is some evidence in table 1, column 15, that the soluble nitrogen increased at the eyes after treatment, but the data in table 4 do not show increases for any of the forms of nitrogen for which analyses were made. Experimental work relating to other forms of nitrogenous substances in the soluble portion would be desirable.

### **Chemical Composition of Tissue-at-eye as Compared with Tissue-not-at-eye**

The analyses in tables 1 and 2 permit a comparison of the chemical composition of the eye-tissue with that of the tissue represented by the

TABLE 4. *Forms of Soluble Nitrogen in Treated and Check Lots of Potato Tissue*

Lot No.	Location of Tissue *	Days After Treatment	Percentage Composition on the Basis of the Fresh Weight of the Tissue									
			Ammonia Nitrogen (N)		Amide Nitrogen (N)		Amino † Nitrogen (N)		Basic ‡ Nitrogen (N)		Non-Basic § Nitrogen (N)	
			Check	Treated	Check	Treated	Check	Treated	Check	Treated	Check	Treated
5	At eye	2	0.006	0.004	0.017	0.007	0.043	0.043	0.043	0.033	0.057	0.044
"	"	4	0.005	0.005	0.012	0.011	0.047	0.053	0.045	0.041	0.057	0.068
"	Not at eye	2	0.008	0.005	0.016	0.021	0.067	0.060	0.039	0.030	0.071	0.058
"	"	4	0.007	0.006	0.013	0.019	0.065	0.070	0.037	0.040	0.074	0.083
11	Whole seed-piece	6	0.005	0.004	0.022	0.019	0.062	0.074	0.040	0.036	0.079	0.074
12	"	6	0.005	0.005	0.035	0.035	0.075	0.085	0.032	0.042	0.098	0.115
13	Not at eye	2	0.004	0.005	0.035	0.036	0.093	0.090	0.033	0.043	0.105	0.104
"	"	4	0.005	0.004	0.035	0.032	0.090	0.092	0.034	0.038	0.100	0.110
"	"	6	0.004	0.005	0.030	0.034	0.079	0.091	0.038	0.050	0.085	0.104

*Note:* The lot numbers, varieties, and treatments correspond to those in tables 1 and 2.

\* See text and foot-notes in tables 1 and 2 for description of type of tissue represented in samples.

† Determined gasometrically by Van Slyke apparatus.

‡ By Kjeldahl method on the phosphotungstic precipitate.

§ By Kjeldahl method on the phosphotungstic filtrate.

rest of the seed-piece. In order to make clear the differences that exist between the composition of the tissue in these localities in the tuber, table 5 has been prepared, in which only the check tissue data in tables 1 and 2 were used for making the comparison. The difference in percentage composition of tissue-at-eye and tissue-not-at-eye has been expressed as a percentage of the value of the tissue-not-at-eye. In this connection it should be borne in mind that the eye-tissue referred to in this experiment consisted of samples obtained by picking out the eyes of seed-pieces, taking about one-tenth gram of tissue at each eye (necessarily taking some of the surrounding tissue along with the eye itself), while the tissue-not-at-eye

TABLE 5. *Comparison of Tissue-at-eye and Tissue-not-at-eye with Respect to Chemical Composition*

Lot No.	Days after Treatment	Difference Between Tissue-at-eye and Tissue-not-at-eye Expressed as Percentage of Tissue-not-at-eye. (+) = Tissue-at-eye Higher than Tissue-not-at-eye; (-) = Tissue-at-eye Lower than Tissue-not-at-eye					
		Soluble Solids %	Reducing Sugar %	Sucrose %	Starch %	Insoluble N %	Soluble N %
1	5	0	+ 5	+15	-17	+108	-27
2	5	+16	- 83	+ 4	+ 7	+ 73	-34
3	2	+32	- 17	- 1	- 5	+ 48	-22
4	5	+25	+ 3	-23	- 2	+ 52	-29
5	2	+27	+310	-44	- 7	+ 32	-31
	4	+33	+230	- 2	- 2	+ 13	- 7
6	3	+34	+ 15	- 2	-18	+ 32	-15
7	2	+21	+ 65	+ 5	- 9	+ 42	+ 1
	5	+19	+ 2	+65	-17	+ 33	+10
8	3	+38	+ 51	-12	-16	+ 34	-23
9	2	+20	+ 67	-19	- 5	+ 60	-27
	4	+ 8	+ 54	-49	+ 9	+ 50	-26
10	3	+12	- 23	-25	- 5	+ 28	-23
Average		+22	+ 52	- 7	- 7	+ 47	-19

Note: Lot numbers, varieties, and treatments correspond to those in previous tables.

is represented by the rest of the seed-piece (about 25 grams of minced tissue). The values for the composition of the not-at-eye portion, therefore, are such values as one would obtain in an ordinary analysis, since the comparatively small amount of eye-tissue (0.1 gram per seed-piece) would not have much influence on the total value.

The values given in table 5 show the amounts in percentages by which the eye exceeds the rest of the seed-piece if the plus sign (+) is given, or the percentage decrement if the minus sign (-) is given.

It will be seen from table 5, columns 3 and 7, that the soluble solids were about 22 percent, and the insoluble nitrogen was about 47 percent, higher in the eye-tissue than in the rest of the seed-piece. The starch, however, was about 7 percent, and the soluble nitrogen about 19 percent, lower at the eye-tissue as compared with the not-at-eye-tissue. The

reducing sugar difference shows a value of 52 percent increase for eye-tissue over not-at-eye, but the variations in this constituent were so large that the results on this point are left in doubt.

The data in table 5, column 5, indicate that the cane sugar is about 7 percent lower in the eye-tissue than in the rest of the seed-piece, but when the variation in different lots is taken into account this difference is not found to be significant statistically. But if the sucrose data for the treated lots in tables 1 and 2 are considered from the same viewpoint, it is found that sucrose was about 35 percent higher in the eye than in the balance of the seed-piece, and this difference is significant statistically. This fact emphasizes the fact previously established: that the most striking effect of the chemical treatment is to cause a large increase in sucrose, especially in the vicinity of the eye.

Table 5 shows that the composition of the eye-tissue differs from that of the rest of the seed-piece, at least with respect to certain constituents, especially starch and nitrogenous substances. But the data do not show how steep is the gradient of change in composition from the eye inward toward the pith, and laterally toward other localities in the cortex. Information on this point would be desirable in arranging to take samples of eye-tissue for it would show whether great care would need to be taken in excluding the tissue adjacent to the eye as completely as possible. It would be possible to obtain samples of eye-tissue that included less of the adjacent tissue than has been done in these experiments if it were necessary, especially if determinations of only certain chemical constituents were made.

It is possible that this difference in composition of tissue-at-eye and tissue-not-at-eye has been a factor in these experiments, and that certain inconsistencies of the data are the results of having included in the sample more or less of the adjacent tissue in one or the other of the samples of comparable pairs.

#### SUMMARY

1. Previous experiments had shown that treatments of dormant potato tubers with ethylene chlorhydrin ( $\text{ClCH}_2\text{CH}_2\text{OH}$ ) and sodium thiocyanate ( $\text{NaSCN}$ ) were effective in breaking the rest period, and these experiments were undertaken to determine what chemical changes are induced in the tissue shortly after the application of the treatments.

2. The chemical composition of treated and check tissue was determined at intervals of 2, 3, 4, 5, and 6 days after treatment, the comparisons not being carried beyond the stage at which sprouts became visible.

3. A partial localization of the changes within the tuber was obtained by making separate analyses of the tissue-at-eye (obtained by picking out about 0.1 gram of tissue from each of about 350 seed-pieces at each sampling period) and of the tissue-not-at-eye (obtained by peeling the seed-pieces after removal of eye-tissue and sampling the remaining tissue after mincing).

4. The most noteworthy difference in composition between treated and



check tissue was in the sucrose content. The treated tissue in every case was higher in cane sugar than the corresponding check, the increase over the check being about 100 percent.

5. Other important differences observed were: (a) an increase in the solids soluble in 50-percent alcohol, the increase in the treated lots over the checks being about 15 to 20 percent; and (b) a decrease, amounting to 2 to 8 percent, in the starch content.

6. These changes were found to have occurred at least within 48 hours after the treatments were applied.

7. The changes were found to be greater in the tissue-at-eye than in the rest of the tissue in the seed-piece, corroborating previous observations that dormancy is localized in the bud tissue, and suggesting the probability that the very first changes occur in the growing-point itself.

8. The other constituents for which analyses were obtained, *i.e.*, reducing sugars, insoluble and soluble nitrogenous substances (50-percent alcohol being the solvent), ammonia, amides, amino acids, basic and non-basic nitrogen, did not show consistent differences between treated and check tissue.

9. No difference between the moisture content of treated and check tissue was observed.

10. The composition of the tissue-at-eye was found to differ from that of the tissue-not-at-eye with respect to certain constituents. The tissue-at-eye was higher in soluble solids and insoluble nitrogen, and lower in starch and soluble nitrogen. The reducing sugar data were inconclusive. Sucrose values tended to be lower at the eye in the check samples, but in the treated samples the sucrose was definitely higher in the eye tissue than in the balance of the seed-piece.

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## THE KILLING OF PLANT TISSUE AND THE INACTIVATION OF TOBACCO MOSAIC VIRUS BY ULTRA- VIOLET RADIATION<sup>1</sup>

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### INTRODUCTION

Radiation from quartz tube mercury vapor arcs and similar sources rich in ultra-violet of wave length shorter than  $290\text{ m}\mu$  are injurious to plant tissue. The purpose of this investigation was to determine what region of the ultra-violet is most injurious, and whether that region near wave length  $290\text{ m}\mu$ , the extreme limit for solar radiation, is injurious to plant tissue.

The investigation was prompted partly by reports that solar radiation as received at high altitudes in Colorado and elsewhere is injurious to plant tissue on certain days when the atmosphere is especially clear and free from clouds. Plants growing in greenhouses appear to be protected. This protection may be due to the absorption of the extreme ultra-violet or to a diminution of the total intensity on passing through glass, which amounts to at least 20 percent.

In most of the work a series of five glass filters supplied by the Corning Glass Works was used to absorb progressive increments of the extreme ultra-violet radiation between wave lengths 200 and  $365\text{ m}\mu$ .

In general it was found that the amount of injury to plant tissue increases rapidly with decreasing wave length between  $290$  and  $200\text{ m}\mu$ . Radiation from the quartz tube mercury arc without a filter will cause a rapid killing of the whole upper surface of young tomato seedlings in one minute as exposed in this investigation. A filter transmitting one percent at wave length  $249.7\text{ m}\mu$  will protect the plant for about 10 minutes on single exposures. The injury can be produced by much longer exposures at longer wave lengths. It is possible to choose suitable filters which will protect plants against injury for 6 hours and yet get marked injury when this time period is doubled in a single irradiation of 12 hours.

These effects are not cumulative. That is, a plant which is only slightly injured by a single 10-minute irradiation through a certain filter will receive very little further injury when irradiated through that same filter each day

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for several weeks provided that each single exposure never exceeds 10 minutes. Evidence is also presented to show that the time for producing a similar injury is decreased in proportion to the increase in incident total radiant energy applied from the quartz tube mercury lamp.

Further investigations are reported on the inactivation of the virus which causes tobacco mosaic using the unprotected quartz tube mercury arc as a radiator. It was found that this virus could be inactivated when exposed through quartz plates in about 15 seconds while 5 seconds exposure greatly reduced its potency. The virus was also inactivated by short exposures when tobacco plant leaves were irradiated immediately after the virus was rubbed over the surfaces, but if irradiated the next day there was little or no inactivation.

#### DISCUSSION OF LITERATURE

Siemens (16) in 1880 observed that the radiation from a carbon arc was injurious to plants. Melon and cucumber leaves, when exposed at a distance of one meter or less, rolled up at the edges and assumed a scorched appearance. He avoided this difficulty by placing the plants farther away from the lamp. Deherain (6) shortly afterward showed that glass protected against this injury by cutting off the most refrangible rays.

More recent work has shown that radiation from a quartz tube mercury vapor arc is similarly injurious to plant tissue. Maquenne and DeMoussy (14) in 1909 showed that such radiation caused a blackening of the leaves and death of the protoplasm of surface cells. Ursprung and Blum (18) in 1917 irradiated a great number of plants and observed the percentage of cells killed in different layers of cells of leaf tissue. A decision on when a cell was dead was based on its power to deplasmolize in distilled water after it had previously been placed in a cane-sugar solution. They found that some plant cells were killed easily while others were much more resistant. Green algal filaments were killed completely in 10 minutes exposure while the cells of agave leaves were not all killed even with 6 hours exposure. It was shown that the amount of killing increases with exposure time and that penetration of leaf tissue depends both on the nature of epidermal and other cell layers and on their number and thickness. These authors noted also that glass plates protect plants against injury from the rays and conclude that wave lengths shorter than  $290\text{ m}\mu$  cause the injury. Since ordinary glass transmits to only  $312\text{ m}\mu$  there is apparently no experimental basis for this conclusion.

Lipperheide (13), using uviol blue glass as a filter, stated that radiation of wave length shorter than  $280\text{ m}\mu$  produces very marked injury on plants. No transmission curves or other similar data for this kind of glass were included. Delf and his associates (7) have done considerable work on the injurious effects of ultra-violet radiation on plants. They observed that exposures of a few seconds (4 to 30) each day would cause most of the

leaves to die and fall off of *Coleus* and *Pelargonium* plants and that there was no definite increase in resistance against the injurious radiation when exposures were increased gradually. Stunting of plants was produced with exposures of one minute or more. Exposures of 30 seconds in the case of *Trifolium* seedlings gave no stunting and a favorable after-effect. In general the presence of pigments gave increased absorption and more injury, although this was not always true. The effect of ultra-violet radiation upon leaves is summarized by Delf as follows:

- (1) Absorption of rays by epidermis and cuticle.
- (2) Latent period without visible changes.
- (3) Lethal effect in epidermal cells (apparent on account of a peculiar "shine" or varnished appearance).

Eltinge (10) observed that a series of commercial glasses, Quartzlite, Vita, and window glass, did not transmit injurious rays from a quartz-tube mercury-vapor arc. The ultra-violet component transmitted by the Vita glass used (limit at wave length 289  $m\mu$ ), seemed beneficial to some plants but without visible effect upon others. The radiation from the open arc (without filter) produced great injury or death with exposures of 30 seconds the first day and an increase of 30 seconds on each succeeding day of irradiation exposed at a distance of 50 inches from the lamp, showing that there was little immunity developed against the injurious rays. When irradiated at 100 inches slightly less injury was observed.

Smith (17) observed that the "biological rays" obtained from an old quartz mercury lamp masked the symptoms of the mosaic disease of tobacco plants. This disease can be transferred from diseased plants to healthy plants by inoculations with expressed and coarsely filtered plant juice. The virus contained in the expressed juice was inactivated by an exposure of 30 minutes to the rays of a quartz mercury vapor lamp. Mulvania (15) reported that this virus could be killed by a similar exposure of one hour. Both of these workers conclude that the virus is not as susceptible to ultra-violet injury as bacteria, since most bacteria are killed when exposed in a clear solution free from air bubbles in less than 60 seconds. Ellis and Wells (9) have published a table showing the exposure time necessary to kill various bacteria. Smith and Mulvania believe further that this resistance to ultra-violet indicates that the virus is a non-living substance similar to an enzym.

Coblentz and Fulton (4) have accurately determined the region lethal to bacteria as well as the relative effectiveness in this respect of different wave lengths in the ultra-violet region. They conclude that a germicidal action is produced by ultra-violet radiation from the Schumann region back to and including wave length 365  $m\mu$ . This includes a part of the extreme region transmitted by window glass and also much of the solar ultra-violet region, and we should therefore expect solar radiation both winter and summer to have some germicidal action even when transmitted through

ordinary glass. The lethal action of wave lengths longer than  $305\text{ m}\mu$  emanating from a quartz mercury arc was found very slow in comparison to the region around  $280\text{ m}\mu$  even though the latter was of much lower intensity. It was estimated that wave lengths shorter than  $280\text{ m}\mu$  were at least 10 times as rapid as those longer than  $305\text{ m}\mu$ . The killing effect was found to be cumulative with no stimulating effect upon growth and no apparent continuation of the lethal action during rest from irradiations. The energy required to kill a bacterium in the most active killing region ( $170\text{--}280\text{ m}\mu$ ) was very small and was of the order of  $4.5 \times 10^{-12}$  gram calories. A further deduction was that in order to produce a rapid killing the radiant flux must exceed a threshold value of about 25 microwatts per square millimeter which obtains at a distance of about 15 centimeters from a 110-volt quartz mercury arc consuming 320 watts.

That this lethal action extends into and probably increases in the Schumann region is indicated by the work of Bovie (1). The lethal action of the Schumann region on green algal filaments and fungus spores he observed to be cumulative and to increase with decreasing wave length. The component of wave length shorter than  $170\text{ m}\mu$  was 15–20 times more destructive than the component of longer wave length.

#### APPARATUS AND METHODS

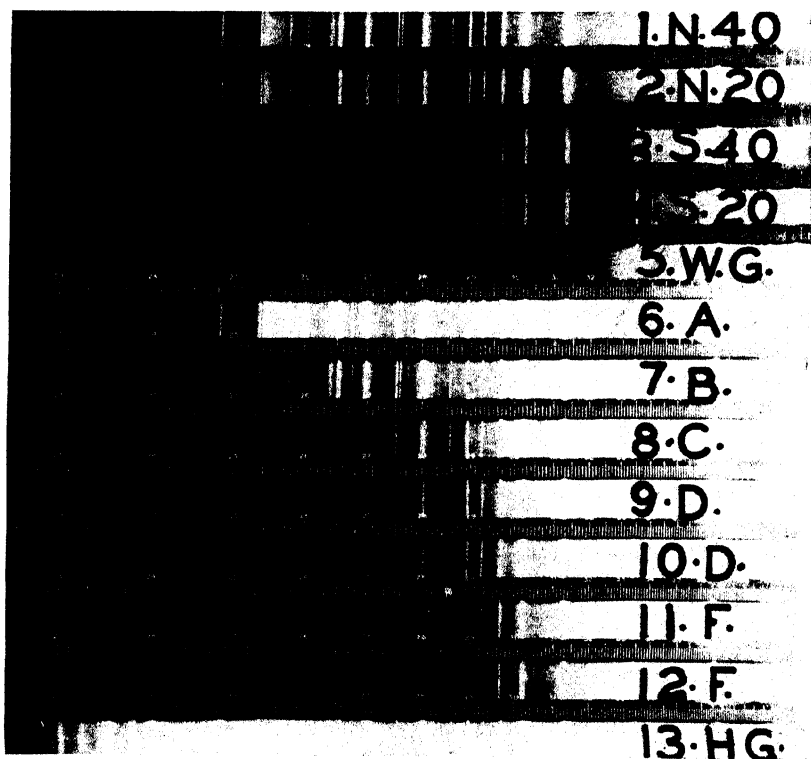
In most of the present work a Cooper Hewitt 220-volt horizontal Uviarc lamp was used. This lamp consumes about 730 watts (160 volts, 4.6 amperes) at the arc after the first 10 minutes of burning. A rectifier and transformer is supplied by the manufacturer so that the lamp may be operated on a 110-volt alternating current line. Since this burner becomes slowly solarized through use over a long period of time a second burner was kept as a standard source of radiation for comparison from time to time. Some experiments were also made with the iron arc as a source of radiation. The effect was similar to the mercury arc but on account of a lower intensity in the ultra-violet region and the difficulty of maintenance this source was abandoned early in the study.

The mercury lamp used is equipped by the manufacturer with a metal shield which completely encloses the quartz tube burner. The metal face of this shield is easily removed or attached by manipulating a spring catch arrangement. A square opening  $6 \times 6$  inches is cut in the center of this face, and a system of metal cleats and spring clips is provided around the edge of the opening for holding glass filters or metal kits which can be used to regulate the size of the opening. In all of the present work glass filters  $6\frac{1}{2} \times 6\frac{1}{2}$  inches were used to cover the opening and the dimensions of the opening effective in transmitting radiation from the lamp was therefore  $6 \times 6$  inches.

In some of the experiments a plano-convex quartz lens,  $4\frac{1}{2}$  inches in diameter and  $8\frac{1}{2}$  inches focal length, was used to concentrate the rays from

the arc. This lens was fitted into a metal plate which could be easily attached to the face of the shield and which, when attached, held the plane face of the lens firmly pressed against the glass filter.

For the most part glass filters were used. The work was started with some pieces of Corex *A* which had previously been used as a roof covering



TEXT FIG. 1. Transmission spectra.

1. Corex *A* plates before solarization, 40 seconds exposure
2. " " " " " 20 " "
3. " " " after " 40 " "
4. " " " " " 20 " "
5. Window glass, 20 seconds exposure
6. Filter *A*, 20 seconds exposure
7. " *B*, " " " "
8. " *C*, " " " "
9. " *D*, " " " "
10. " *D*, 10 " " "
11. " *F*, 20 " " "
12. " *F*, 10 " " "
13. Open arc without filter, 10 seconds exposure

on one of the greenhouses. It has been noted previously in a Bureau of Standards publication (2) that this glass does not solarize, or lose its transmission appreciably in the ultra-violet region, when exposed to sunlight for 14 months. The samples of the glass used in these experiments were found to transmit faintly as far as wave length  $225\text{ m}\mu$ . When tomato plants were exposed through them to rays from a quartz mercury arc a definite killing effect was produced in a comparatively short time. It was found, however, that these filters solarized so rapidly that after an exposure of one hour plants could be exposed through them for an additional hour with little or no injury to tissues. The filters after such an exposure transmitted faintly to wave length  $240\text{ m}\mu$  although the whole region of wave length shorter than  $290\text{ m}\mu$  was dimmed appreciably. After complete solarization (8 hours exposure to the lamp) the filters still transmitted to wave length  $248\text{ m}\mu$  (text fig. 1). Following these experiments an effort was made to get filters which would transmit various parts of the region between wave lengths  $290\text{ m}\mu$  and  $225\text{ m}\mu$ . It was found that several layers of gelatin could be deposited on quartz plates to produce filters transmitting to  $250\text{ m}\mu$  or slightly beyond. These filters had the disadvantage of not holding up well under prolonged irradiation from the lamp due mainly to the heat involved. Mica filters were also tried but the thinnest piece measured did not transmit much beyond wave length  $290\text{ m}\mu$ .

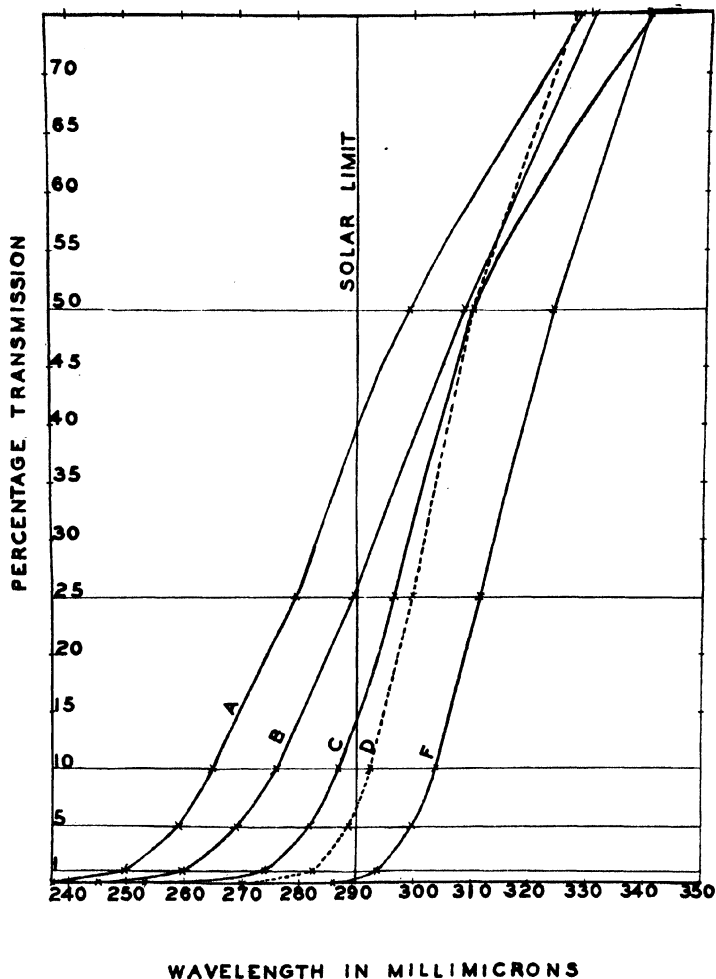
A series of glass filters was finally obtained through the coöperation of the Corning Glass Works. These filters have a high transmission in the ultra-violet region and do not further solarize upon prolonged irradiation with the quartz mercury arc. The limits of transmission of this series of glasses in the ultra-violet are shown in text figure 1. The spectrograms were taken on a Hilger quartz spectrograph. After many of the experiments reported herewith had been completed the filters were sent to the Bureau of Standards and the percentage transmission of each filter between wave lengths 249 and  $349\text{ m}\mu$  was determined. These measurements were made photographically by means of a Hilger Sector photometer. The method has been described elsewhere (3). The percentage transmission of each filter is shown plotted against wave length in text figure 2 and is based upon this data.

The incident total radiant energy 15 inches from the lamp tube, measured by means of a Weather Bureau type pyrliometer (12), was .17 gram calories per square centimeter per minute. When filter *A* was placed over the lamp an energy loss of approximately 15 percent was observed. A further decrease of about 6 percent in total incident energy occurred when either filters *C* or *D* were substituted for *A*. The use of the quartz concentrating lens in connection with filter *A* resulted in a concentration of about 3 times the total energy value. Actual readings with the pyrliometer surface 15 inches from the quartz tube burner were as follows:

With filter only, .14 gr. cal.

With filter plus lens, .42 gr. cal.

It should be pointed out that the pyrheliometer used was covered with a hemisphere of uviol glass which transmitted faintly to 253  $m\mu$  before solarization. The transmission in the extreme ultra-violet region would undoubtedly decrease appreciably when exposed to the rays from the



TEXT FIG. 2. Transmission graphs of various filters.

lamp but on account of the return of this instrument for recalibration the actual amount of the decrease has not as yet been determined. A part of the energy loss due to the filters as observed above was no doubt due to the visible component as well as the ultra-violet.



## EXPERIMENTS WITH PLANTS

After a preliminary study of the effects of the ultra-violet region on 5 or 6 different species of plants the tomato was selected as a subject for more detailed observations. Small plants  $2\frac{1}{2}$  to 3 inches high were used in most of the work. In testing the cumulative effects of irradiation, where daily irradiations were given over a considerable period of time, the plants often grew to a height of 12 to 15 inches. Such a series was transferred to larger pots from time to time so as to maintain a healthy normal growth of the control plants. No great difference in the amount of injury could be determined between young plants with the first pair of leaves (not counting the cotyledons) and large plants 8 to 12 inches high. Old leaves appeared to be slightly more resistant than young leaves just open. Also no great difference was observed when the plants were one inch closer or one inch farther away from the lamp during the exposure, but it was established that 6 inches farther away made a slight decrease in the amount of injury. All plants were irradiated during the study at a distance of approximately 15 inches from the nearest point on the quartz tube to the tip of the plant.

## RESULTS OF IRRADIATION

It was soon established that the amount of injury increased rapidly with decreasing wave length from 290  $m\mu$ . The open arc (without a filter) caused considerable injury in 30 seconds. When the most transparent filter (*A*) was used no great injury could be detected until the plant was exposed for about 15 minutes in a single exposure, while it required approximately 60 minutes to produce as severe an injury as 30 seconds exposure to the open arc. The transmission of the filters is shown in table 1 and text figures 1 and 2. No injury was produced when plants were irradiated through filter *D* until the continuous exposure period exceeded 6 hours, while 12 hours continuous exposure to the radiation transmitted by this filter gave less injury than 30 seconds exposure to the open arc. Using the quartz concentration lens as already described with this filter an injury could be produced in  $3\frac{1}{2}$  to 4 hours single irradiation which was about the equivalent of 12 hours exposure without the lens. Since pyrheliometer measurements of the relative total energy in the two cases were about 3 to 1 it is interesting to note that the injurious effect produced is approximately proportional to the amount of energy received when the same wave lengths are transmitted and that a certain "minimum dose" or threshold value of energy is necessary to produce any injury, the amount of which depends upon wave length.

The effect of exposures made once each day or every second day through the various filters was studied over periods varying from 2 weeks to 45 days. Using the most transparent filter (*A*) it was observed that 10 minutes exposure each day or every second or third day caused very little more local injury than a similar single exposure. As the leaf tissue grew,

however, and more new surface was exposed each day normal to the rays the injurious effect on the whole plant was greater and more certain for observational purposes. In no case, however, could a more injurious effect be produced with a much shorter daily exposure over a period of weeks than could be produced with a single exposure. The effect is, therefore, not cumulative locally, but additive to a certain maximum degree when the whole aerial portion of the plant is considered.

In Plate XXV, figure 2, is shown the effect of irradiating young tomato plants for periods of 10 and 15 minutes per day during the period September 6 to October 22 through filter *A*. The plant at the left is the control. The center plant received 10 minutes exposure and the plant at the right 15 minutes. The plant receiving only 10 minutes exposure is slightly injured while the one receiving 15 minutes per day is badly injured. Both plants received 36 separate periods of irradiation of 10 and 15 minutes, respectively. A single exposure of 15 to 20 minutes also produced severe local injuries to parts of leaves exposed normal to the rays but the injury to the whole plant was much less severe since the total surface presented in a single exposure is small. The energy transmitted from the lamp through this filter does not cause any visible injury to tissue when single exposures do not exceed 5 minutes per day. With this filter, as is the case with all other filters used in the study, there is a minimum or threshold value of energy which must be exceeded to produce an injury. As the region transmitted moves so as to exclude rays of shorter wave length the time necessary to produce injury rapidly increases, until a point is reached where insufficient energy is transmitted to produce any injury even after prolonged irradiation.

The minimum exposure time necessary for producing marked injury was determined for the series of 5 filters mentioned above. This was accomplished by irradiating young plants each day or every other day for periods sufficiently long to produce maximum injury for that particular exposure period and filter. The data is presented in table 1. Photographs of the plants irradiated in this series of experiments are shown in Plate XXV, figures 1, 2, and 3, and Plate XXVI, figure 4. These plants show, therefore, all of the cumulative injuries or additive effects which it is possible to obtain with the same energy source and the respective filters and exposure periods. It is apparent from this data that the open arc produces a marked injury in exposure periods of 30 seconds. Figure 1 shows the control and plants which have been exposed for 30, 60, and 120 seconds, respectively, at each of 11 irradiations from October 5 to 22. Lines in the spectrogram of this arc can easily be seen to wave length  $220\text{ m}\mu$ . When plants are exposed for 15 minutes through filter *A* a similar injury is produced, but not quite as severe as 30 seconds exposure to the open arc. The plants are shown in figure 2. From left to right are the control plant and plants exposed for 10 and 15 minutes, respectively, in each of 36 separate irradi-

ations from September 6 to October 22. This filter transmits one percent of the incident radiation at wave length  $249.7\text{ m}\mu$  and has an extreme limit of transmission at  $237\text{ m}\mu$ . The plants shown in figure 3 received 15 irradiations of 30, 45, and 60 minutes, respectively, through filter *B* during the period September 18 to October 19. The control plant is at the left. The plant receiving 30 minutes exposure is very slightly injured. The one receiving 60 minutes is severely injured. Plants may be exposed repeatedly through this filter for 25 minutes each day with no visible effect. Radiations transmitted through filter *C* cause severe injury after 3 hours exposure. These plants were photographed after 7 periods of irradiation of 1, 2, and 3 hours, respectively, during a 10-day period and are shown in Plate XXVI, figures 4 and 5. Two hours exposure produces almost no injury while 3 hours gives a severe injury. The plant is protected completely when each single exposure does not exceed  $1\frac{1}{2}$  hours. This filter transmits one percent of the incident radiation at wave length  $273.7\text{ m}\mu$  and has an extreme limit at  $253\text{ m}\mu$ . Using filter *D* no injury was produced after two periods of irradiation of 6 hours each. Single irradiations of 12 hours produce considerable injury. As pointed out above, a similar injury could be produced when the quartz concentration lens was placed over this filter in single exposures of  $3\frac{1}{2}$  to 4 hours. This filter transmits one percent of the incident radiation at wave length  $281.1\text{ m}\mu$  and has an extreme limit at  $270\text{ m}\mu$ . The leaf injury produced by  $3\frac{1}{2}$  hours irradiation with both the filter and lens, together with 12 hours irradiation with the filter alone, are shown in figure 6. No injury could be produced on plants taken from a greenhouse and irradiated for long periods through filter *F*. The concentrating lens was used in conjunction with this filter for a period of 18 hours continuous exposure with no visible injury. This is the equivalent of 54 hours irradiation without the lens. This filter transmits one percent at wave length  $293.5\text{ m}\mu$  and has a transmission limit at  $286\text{ m}\mu$ . The transmission of the filters, the exposure time necessary to produce marked injury, the relative time to produce injury equal to or slightly less than one second exposure to the open arc, and the time of exposure not injurious to plants, are summarized in table 1.

If we assume the killing effect produced in several exposures of 30 seconds each from the open arc as equal to 1, it takes more than 30 units to produce the same effect through a filter transmitting one percent at wave length  $249.7\text{ m}\mu$ , as compared with 120 units through a filter transmitting one percent at  $259.7\text{ m}\mu$ , 360 units at  $273.7\text{ m}\mu$  and 1,440 units at  $281.1\text{ m}\mu$ , while as pointed out above a filter transmitting one percent at  $293.5\text{ m}\mu$  protects plants indefinitely which have been receiving normal solar radiation.

Coblentz and Kahler (5), using a similar quartz mercury arc, have shown that the total amount of ultra-violet radiation of wave length less than  $400\text{ m}\mu$  is about the same for solar radiation as for the mercury arc, that is,

TABLE I. *Relation between Transmission of Various Filters and the Time Required to Produce Marked Injury to Young Tomato Plants Using a Mercury Arc in Quartz as a Source of Radiation. Plants Taken from Greenhouse and Irradiated Immediately*

Filter Number	Open Arc	A	B	C	D	F
Thickness (millimeters).....	—	1.9	3.11	3.99	2.0	5.06
Wave length $m\mu$ at which glass transmits 1%.....	—	249.7	259.7	273.7	281.1	293.5
Extreme limit of transmission $m\mu$ .....	200	237	245	253	270	285
Time required to produce marked injury.....	30 secs.	15 mins.	1 hr.	3 hrs.	12 hrs.	54 hrs. No effect
Relative time to produce injury equal to or slightly less than 30 seconds exposure to open arc.....	1.0	30.0 5 mins.	120.0 25 mins.	360.0 1 $\frac{1}{4}$ hrs.	1,440.0 6 hrs.	Protects indefinitely Protects indefinitely
Time of exposure not injurious to plant..	—					

about .02 gram calories per square centimeter per minute. The energy distribution (they point out further) is very different, since sunlight terminates at about 300  $m\mu$  at near sea level while the mercury arc has 5 percent of its total radiation, or 20 percent of the ultra-violet component, of wave length shorter than 300  $m\mu$ . This being the case, since no injury is produced by energy transmitted through filter *F* even though it is concentrated threefold, it is thought that no injury can be produced by the ultra-violet component of solar radiation at the normal intensity and range of wave lengths in altitudes where plants grow. This filter transmits to wave length 285  $m\mu$  and transmits one percent at 293.5  $m\mu$ . Since the limit for solar radiation is wave length 290  $m\mu$  and the energy at that wave length must always be extremely small it is believed that it could not possibly cause injury to plant tissue since the threshold value of energy necessary for producing injury at this wave length is comparatively great.

Plants which had been removed from the greenhouse to a dark basement room 3 or 4 days before irradiation were observed to be more severely injured by smaller exposures than those which were taken directly from the greenhouse. A series of two plants so treated together with the control are shown in Plate XXVII, figure 7. The extreme injury of a single exposure of 3 minutes to the open arc is shown on a plant which has been kept in darkness for 4 days. Since plants kept in darkness were found to be more susceptible another attempt was made to produce an injury through filter *F* using the concentrating lens. As pointed out above no injury had ever been produced through this filter on plants which had been exposed continuously to solar radiation, that is, taken from the greenhouse and irradiated at once. The plants were kept in a dark room for 4 days and were irradiated on the fifth for 16½ hours. A slight injury was produced—the characteristic “shine” on one or two leaflets. Plants so treated are apparently very sensitive to the visible region as well as to ultra-violet, since even the control plants showed some evidence of light injury upon return to greenhouse conditions, but none of the “shine” or varnished appearance which is characteristic only of ultra-violet injury. This injury was produced by wave lengths longer than 285  $m\mu$ . The fact that the characteristic ultra-violet injury can be produced very near the extreme limit for solar radiation (wave lengths longer than 285  $m\mu$ ) is interesting, since it shows that the limit of the energy range for plant growth is definitely fixed in the ultra-violet region, and that one needs to overstep the limit of solar radiation as the plant ordinarily receives it by only an extremely narrow margin to cause marked injury to tissues. Since the conditions under which this injury was produced through filter *S*1–5.06 (4 days in darkness followed by the equivalent of approximately 50 hours irradiation from the quartz mercury arc) are not found in nature, the earlier conclusion, that the ultra-violet component of solar radiation as the plant receives it at high altitudes cannot be injurious, is still believed to be correct.

The effect of the shorter wave lengths (ultra-violet, Schumann region, and X-rays), where absorbed by plant tissue, is in general injurious. It may lead to stimulation of growth or even possible change in genetic constitution. So far as our own observations go in the effect of the ultra-violet region there is no marked stimulation but only injury. Plants which have received repeatedly such exposures which do not injure tissue show no well defined increases in growth or fruit production or other stimulation. This work is being continued, however, in a study which will be supplemented by a chemical analysis of tissue which has been exposed repeatedly to various regions of the ultra-violet component of radiation from a quartz mercury arc.

#### OBSERVATIONS ON THE KILLING OF THE VIRUS WHICH CAUSES TOBACCO MOSAIC

Since it was observed in the foregoing researches that plants were able to withstand radiation of wave length shorter than  $290\text{ m}\mu$  for a considerable time with no apparent injury provided a certain threshold value of energy was not reached, and further since Coblentz and Fulton (4) had observed that wave lengths as long as  $365\text{ m}\mu$  had a germicidal effect on bacteria, it might appear that there was some possibility of killing the tobacco mosaic virus within living plant tissue, assuming that the virus would be as easily killed as bacteria. Experiments were therefore made to determine, first, the exposure necessary to kill the virus when purified and exposed to ultra-violet radiation in a water solution in thin layers between quartz and glass plates; and second, whether the virus could be killed within plant tissue. Samples of virus were prepared by Dr. C. G. Vinson by precipitation with acetone in accordance with a method already described by him (19). Tests of the potency of the virus were made by Dr. F. O. Holmes using a method which he has described elsewhere (11). This consisted briefly in transferring the virus to be tested to leaves of *Nicotiana glutinosa* by rubbing lightly with a piece of cheesecloth moistened with the virus solution. The potency is indicated by the number of colonies of dead cells or circular brown lesions which develop on the leaves after a period of 2 to 3 days incubation. The procedure of irradiation with the quartz mercury lamp was in detail as follows: 6 drops of the purified virus were distributed by means of a pipette over an area 2 to 3 inches square on a glass plate. This was covered with a quartz plate  $\frac{1}{8}$  of an inch thick and the virus was exposed through the quartz plate at about 15 inches from the burner. The quartz plate was then removed and 15 to 20 drops of distilled water were added so as to have sufficient liquid to saturate a small piece of cheesecloth. The irradiated virus was then rubbed lightly over the leaves of the tobacco plant. The first experiment showed that virus could be completely inactivated in exposures of one minute (fig. 8). Controls protected by window glass or a Noviol O filter (transmitting to wave length 312 and

390  $m\mu$ , respectively) produced great numbers of lesions after being exposed for 10 minutes to mercury arc radiations. Similar exposures of 5, 15, 20, and 45 seconds were made, using virus from the same sample. It was found that 5 seconds inactivated most of the virus while not a single lesion was produced when it was exposed for 15, 25, and 45 seconds. Figure 9 illustrates a single leaf from the control plant (left) and another from a plant inoculated with virus after it had been irradiated for 20 seconds. This virus was prepared from diseased tobacco plants which had been growing in a greenhouse. Further tests upon virus from plants grown in the field during the following summer showed this to be more resistant as one or two lesions were produced from virus which had been irradiated for 30 seconds. This difference was probably due to the protective action of the greater amount of contaminating material and the difficulty of getting a sufficiently pure solution. Even with the additional contaminating material only a very few lesions developed after an exposure of 15 seconds to the open arc.

Both Smith (17) and Mulvania (15) reported previously that the virus of tobacco mosaic could be killed in exposures of 30 minutes to one hour to mercury arc radiation. They concluded that since the virus was observed to be more resistant than bacteria to ultra-violet radiation it is probably enzymatic or chemical in nature. Most bacteria are killed by short exposures of one minute or less (9). Following a similar line of reasoning, since it is shown that the virus can be killed by an exposure of a few seconds, one might conclude that it is entirely bacterial in nature. This does not necessarily follow since enzymes and chemical compounds vary considerably in their resistance to these rays and it is possible to find non-living compounds which are rapidly changed by extremely short exposures. The evidence shows only that the virus, whatever its nature, can be inactivated, when sufficiently free from contaminating material, by an extremely short exposure to ultra-violet radiation.

Further experiments showed that the virus could be killed with a short exposure (1 minute) when spread upon the plant leaf surface if irradiated at once. If irradiated the day following inoculation there was no appreciable killing of the virus. It is apparently impossible to inactivate the virus when it has penetrated far into plant tissue, although irradiations were given of sufficient intensity and quality to kill the whole upper surface of the plant leaves.

#### SUMMARY

1. Using a mercury-vapor arc in quartz as a radiator and a series of filters, the time required to produce marked injury on young tomato plants has been determined. The filters absorb progressive increments of the extreme ultra-violet component of radiation from this lamp toward wave length 290  $m\mu$  (the extreme limit for solar radiation).

2. The time of exposure necessary to cause injury was observed to

increase rapidly as more and more of the extreme ultra-violet component was absorbed. Filters which transmit one percent of the incident energy at wave length  $281.1\text{ m}\mu$  were found to cause marked injury to plants only after 12 hours irradiation while the open arc (without a filter) produced a more severe injury in 30 seconds exposure.

3. It was determined by means of a quartz concentrating lens that apparently the time of exposure necessary to cause marked injury is approximately inversely proportional to the incident energy, using the same quality of radiation.

4. No apparent injury is produced on tomato plants until a certain "threshold value" of exposure time is reached in a single irradiation. The time necessary to reach this value increases rapidly with increasing wave length so that plants can be exposed repeatedly through a filter transmitting one percent at wave length  $273.7\text{ m}\mu$  for  $1\frac{1}{2}$  hours each day with no apparent injury.

5. The injury produced is not cumulative but since new tissue is presented normal to the incident radiation on each succeeding day of exposure the total injurious effect on a plant is much greater when plants are exposed each day or every second day for the same time over a considerable period.

6. No typical ultra-violet injury to tomato plant tissue could be produced within the extreme limits of solar radiation, that is, by wave lengths longer than  $289\text{ m}\mu$ , except under conditions which never occur in nature. The injury was produced by irradiation through a filter which transmitted faintly to wave length  $286\text{ m}\mu$ , by exposing young plants continuously for  $16\frac{1}{2}$  hours after the plants had been kept in darkness for 4 days. Since a concentrating lens was used to produce this result which increased the total intensity threefold, approximately 50 hours continuous exposure would be required to produce the same result without the lens. It should be pointed out further that whenever the natural range for solar radiation is overstepped by a very narrow margin injury results.

7. No stimulation to increased growth or fruiting was observed in these experiments, but only injury.

8. The virus which causes tobacco mosaic was found to be completely inactivated by 15 seconds exposure to the open arc when the virus was prepared sufficiently free from contaminating material.

9. This virus could not be inactivated when it had penetrated plant tissue.

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## EXPLANATION OF PLATES

### PLATE XXV

FIG. 1. Effects of open arc. Left to right: control, exposed 30, 60, and 120 seconds respectively in each of 11 exposures over a 17-day period.

FIG. 2. Effects of irradiation through filter A. Left to right: control, irradiated 10 minutes and 15 minutes respectively in each of 36 exposures over a 46-day period.

FIG. 3. Effects of irradiation through filter B. Left to right: control, irradiated 30, 45, and 60 minutes respectively in each of 15 exposures over a 31-day period.

### PLATE XXVI

FIG. 4. Effects of irradiation through filter C. Left to right: control, irradiated 1, 2, and 3 hours respectively in each of 7 exposures over a 10-day period.

FIG. 5. Top view of 2-hour (right) and 3-hour (left) plants shown in figure 4 above.

FIG. 6. Irradiated through filter D. Left, leaf exposed for 3½ hours with lens and filter. Right, leaf irradiated for 12 hours with filter only. The path of the rays is indicated on leaf at left with India ink, while region of maximum injury on leaf at right is indicated by X.

### PLATE XXVII

FIG. 7. Irradiated with open arc (without filter). Left, control; center, plant irradiated 3 minutes immediately after removal from greenhouse; and right, plant irradiated 3 minutes after being placed for 4 days in a dark basement.

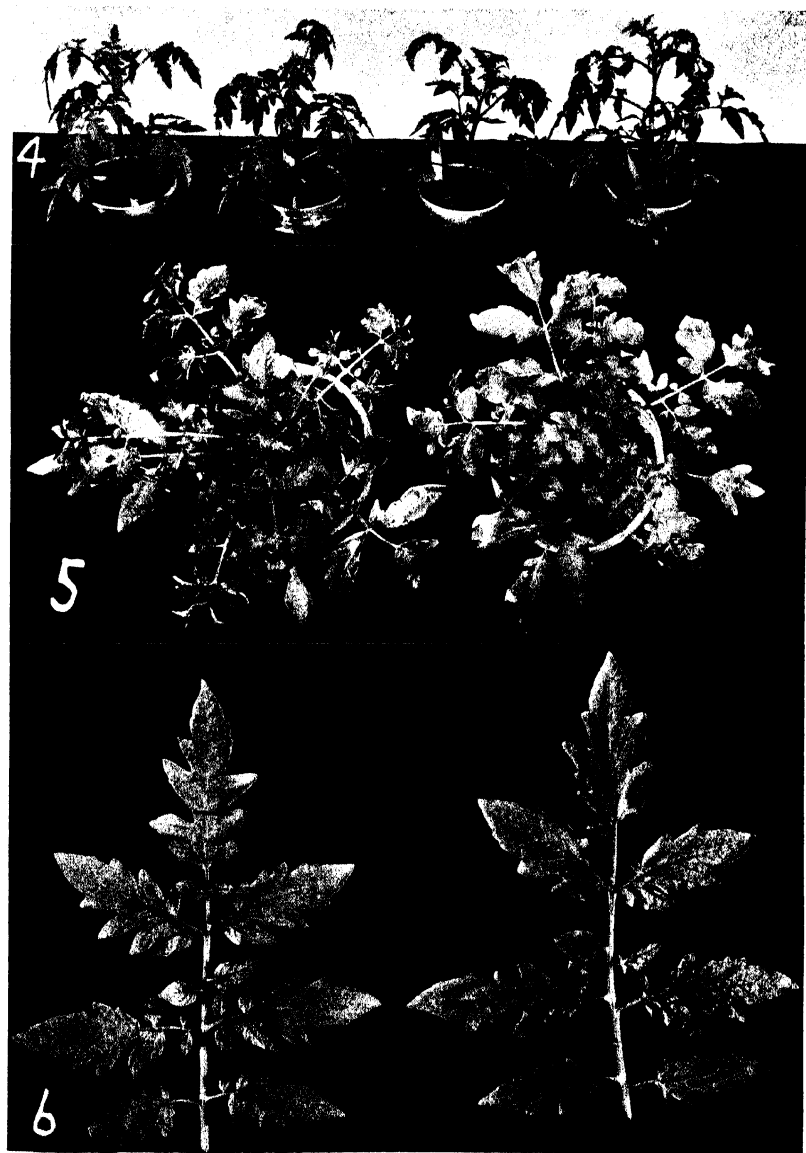
FIG. 8. Tobacco plants which have been inoculated with the mosaic virus which has been irradiated for 1 minute (at left) and 3 minutes (center); and the control plant (at right).

FIG. 9. Leaves from inoculated tobacco plants. Left, from control plant; and right, from plant which had been inoculated with virus which had been irradiated through a quartz plate for 20 seconds.



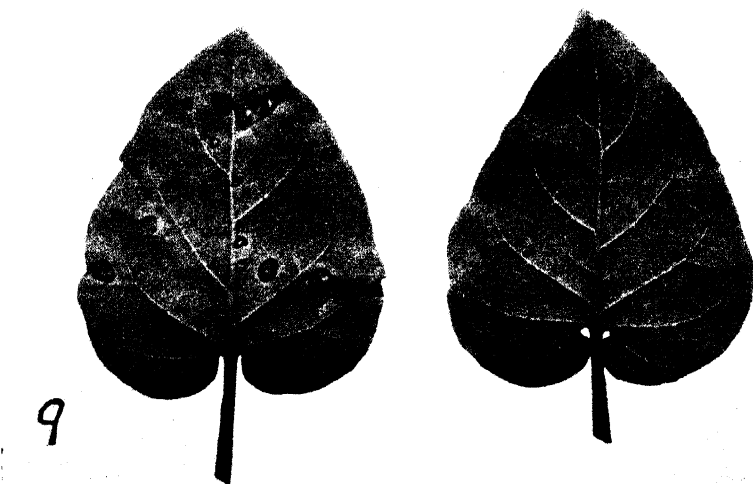
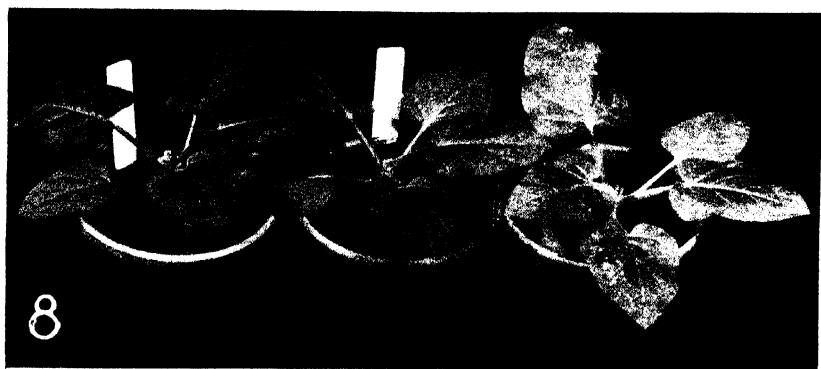
ARTHUR AND NEWELL: ULTRA-VIOLET RADIATION





ARTHUR AND NEWELL: ULTRA-VIOLET RADIATION







## THE INFLUENCE OF LIGHT INTENSITY AND LIGHT QUALITY UPON THE GROWTH OF PLANTS<sup>1</sup>

HARDY L. SHIRLEY

### INTRODUCTION

Since the discovery that green plants can assimilate carbon in the presence of light, light has been recognized as being one of the major factors influencing the growth and other characteristics of vegetation. Plant ecologists have considered light conditions to be one of the important factors in instituting succession. Foresters have based their theories and practices of silviculture upon the light requirements of the forest trees.

When it became possible to measure light conditions under the forest, the decrease in intensity was found to be exceedingly great. Salisbury (24) gave values as low as 0.16 to 1.3 percent of full sunlight in an oak-hornbeam forest. His upper values were about 10 percent. Kvapil and Nemec (18) gave the average intensities under Austrian forests as 2 to 15 percent of outside light. Measurements of the total radiant energy under a 70-year-old mixed hardwood stand near the Boyce Thompson Institute gave values ranging from 0.5 percent in dense shade to 16 percent in sun "flecks." A dense stand of sumach allowed only 2 percent of the energy to pass through. Measurements by other workers indicate that under a well stocked stand the light intensity is usually below 10 percent of outside intensity and often as low as 1 percent. The intensity at any one spot varies greatly from time to time depending upon whether or not a "fleck" of direct sunlight strikes it.

Light under a forest canopy suffers not only a great depletion in intensity but also a change in quality or spectral energy distribution. Knuchel (17) found the light under a beech forest to contain about 12 percent green and yellow, 7 percent red, and less than 5 percent of blue and violet, as compared with zenith skylight in the open. Similar results were obtained for other broad-leaved trees. The change in quality was more pronounced when the sun was shining than when the sun was hidden by clouds. Knuchel found no significant change in the light quality under spruce and fir canopies or other needle-leaved species. The measurement of the light quality under a forest canopy is a difficult problem. Knuchel's measurements seem to be the most reliable ones made to date.

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.



Klugh (15, 16) measured the spectral quality of forest light. He found a spruce-cedar swamp forest to transmit 0.036 percent in the orange region, 0.33 percent in the green, and 3.05 percent in the blue-violet region. The reason for the discrepancy between these two sets of measurements is largely due to the fact that Knuchel compared forest light with skylight, while Klugh compared it with sunlight. Skylight has its maximum intensity in the blue-violet region of the spectrum, while the maximum energy of sunlight is in the yellow-green region (Kimball, 14). In the forest, skylight forms a much higher percentage of the total light than it does outside. The quality of daylight is thus changed in two ways in passing through a leafy canopy. Through partial absorption by leaves the light loses more red and blue than green and yellow. By decreasing the proportion of sunlight to skylight the red region is diminished more than the blue. The latter change seems to be of much greater importance than the former.

In the past there has been a tendency among plant ecologists and foresters to over-emphasize the importance of light at the expense of other factors. Experiments on trenced areas by Fricke (9) and Toumey (28) have clearly demonstrated that survival and growth are often determined by the extent of root competition for moisture and nutrients rather than by light. However, moisture conditions alone are not sufficient to explain why bunch grass and red cedar will grow in dry exposed rock crevices and not in shaded ravines. Root competition does not account for the large numbers of epiphytes and lianas which give the characteristic appearance to the tropical rain forest.

The problem of the light requirements of plants is of importance to the greenhouse man as well as the forester and the plant ecologist. Even the fancier, who tries to grow plants in living rooms or sun porches, is concerned with the light required to get healthy, vigorous growth of his plants.

In the study which follows an attempt has been made to discover what the light requirements for the survival of a few plants are, and how the rate of growth changes with increasing light intensities. A study of the effects of light quality upon growth has also been made, with particular emphasis upon the blue region of the spectrum, as compared with the red.

## DISCUSSION OF LITERATURE

### Effect of Light Intensity Upon Photosynthesis

Blackman and Matthaei (2) and others have shown that with low light intensities the rate of photosynthesis is almost directly proportional to the light intensity if other factors are not limiting. At higher intensities the slope of the curve falls off and approaches a line parallel to the axis, as shown by Boysen-Jensen (4) and Harder (13).

### Effect of Shading on Plant Growth

The effects of shading on plant growth were studied by Combes (6), Garner and Allard (10), Lubimenko (19), Popp (21), Rosé (23), Shantz (27), and Zillich (29).

Lubimenko found that the amount of dry matter produced increased with increasing light intensity up to a certain maximum and then decreased. He states that the optimum intensity for growth increases as the chlorophyll concentration decreases. His light intensities are all expressed in terms of the transmission of a certain piece of glass. *Robinia Pseudo-Acacia*, *Pinus silvestris*, *Fraxinus excelsior*, and *Tilia parvifolia* attained maximum dry weight at intensities corresponding to those which would pass through 7, 9, 9, and 27 layers of the glass respectively. *Helianthus annuus* attained maximum dry weight under full sunlight. Lubimenko states that chlorophyll concentration increases with decreasing light intensity until a certain maximum is attained and then diminishes. Plants having a high chlorophyll concentration have greater power to vary it, and can thus adapt themselves to a wider range of intensities than those which have a low chlorophyll concentration. They also attain maximum dry weight at lower light intensities than plants having low chlorophyll concentration. He found that height and leaf area behaved as dry weight but attained maximum development at lower intensities. The percentage of dry matter usually decreased with decreasing light intensity. He noticed that in general root growth increases and stem growth decreases with increasing light intensity within certain limits. Since no measurements of light intensities were made it is difficult to compare his results with those obtained by other investigators.

Combes (6) found the optimum light intensity for the production of dry matter in plants to increase with increasing age of the plant. Maximum dry weight of fruit always occurred in full light intensity. His optimum intensities are somewhat higher than those given by Lubimenko, but otherwise his conclusions are in general accord. Rosé (23) obtained maximum growth with full sunlight intensity. The shades used by Combes and Rosé were much larger and more satisfactory than those of Lubimenko.

Shantz (27) found the fresh weight of potato, cotton, lettuce, and radish to increase with decreasing light intensity from 50 to 15 percent of full sunlight in Louisiana. None of the plants he tested were able to grow past the seedling stage when the light was reduced to 6 percent of full sunlight. Dry weight determinations were not made.

Garner and Allard (10) found decrease in seed production and in dry weight of tops of soy-beans when grown under shades. Zillich (29) noticed a delay in the time of flowering and fruiting of plants grown under lattice shades. The optimum intensity for green weight was 50 to 75 percent intensity for most of the plants he used. He found that weeds attained greater dry weight under reduced light intensities while cultivated plants

always had maximum dry weight when grown in the open. He also confirmed many of the conclusions of Lubimenko.

Popp (21) obtained the greatest vigor of growth of soy-beans in his unshaded plot, although the shaded plants had longer stems. Dry weight measurements were not given.

In general, shading experiments show that the light intensity cannot be reduced much below 50 percent of full sunlight in temperate regions without causing a decrease in the growth of many plants. In the majority of cases maximum dry weight was produced by plants receiving the full normal sunlight of the region in which they were grown.

### Experiments with Artificial Light

Burns (5) determined the amount of radiation from a Mazda lamp required to maintain a carbon dioxide balance between the plant and the surrounding air. At this intensity the carbon dioxide utilized in photosynthesis would just be equal to that given off in respiration. Comparing this radiation intensity with that from the sun at noon of December 22, at Burlington, Vermont, he found various tree species to have minimum light requirements of from 2 to 17 percent of total solar radiation. In this paper the radiation from neither the lamp nor the sun were given in absolute heat units. Thermopile measurements of total heat energies cannot be used directly to compare artificial light with sunlight in their effects on photosynthesis due to the differences in color temperature or spectral energy distribution of the sources. The carbon dioxide concentrations maintained by Burns in the assimilation chamber were considerably above that of normal air, being on the average 0.5 percent. Since the rate of photosynthesis varies with the carbon dioxide concentration as well as the light intensity, a concentration of 0.5 percent would cause a carbon dioxide balance at lower light intensities than at a concentration of 0.035 percent. It must also be borne in mind that the photosynthetic performance of any plant is influenced not only by the light conditions under which it has been kept during the twenty-four hours preceding the test period, but also upon the light conditions during a much longer preceding period. Rosé (23) showed that plants grown under a low light intensity were able to assimilate carbon dioxide at a much faster rate in low intensities than similar plants which had previously been growing under high light intensities. Short-time experiments upon the rate of gas exchange of plants under artificial conditions are valuable in studying the physiology of the plant, but it seems highly improbable that the results of any such experiments can be applied directly to account for the growth of plants growing under natural conditions.

Grasovsky (11), using the apparatus developed by Burns, found a ~~zero~~ carbon-dioxide exchange with white pine at 174 foot-candles. This is probably about the same intensity found by Burns but is expressed in relative light units.

TABLE I. *Dry Weight Produced Under Different Light Qualities. (Data from Popp, 1926)*

House Number	Wave Lengths Transmitted (millimicrons)	Light Intensity (Percent of Outside Illumination)	Dry Weight per Plant (grams)					Dry Weight per 100-percent Intensity *				
			Sudan Grass	Sun-flower	Tobacco	Tomato	Soy-bean	Sudan Grass	Sun-flower	Tobacco	Tomato	Soy-bean
I.....	312-720	80.0	5.35	26.56	20.60	77.01	2.82	6.7	33.2	25.7	96.3	3.5
II.....	290-720	46.6	5.37	20.11	25.10	86.09	2.58	11.5	43.1	53.9	184.9	5.5
III.....	389-720	66.1	5.01	19.73	21.60	83.43	3.01	8.5	29.9	32.7	126.1	4.6
IV.....	472-720	56.7	3.66	6.06	15.10	66.00	1.98	6.4	10.7	26.6	116.2	3.5
V.....	529-720	37.0	3.76	0.36	13.00	59.09	0.86	10.2	1.0	35.1	160.0	2.3

\* Calculated by the writer.

Bates and Roeser (1) grew coniferous seedlings for a period of nine months under artificial illumination of 10 hours daily duration. The experiment was arranged so that the plants grew at varying distances from the light source. By measuring the radiation intensities under which the plants survived, a minimum light requirement was obtained. This intensity was compared by the use of a thermopile with noon summer sunlight at Colorado Springs, Colorado, for the year of 1926. The method of comparison is open to the criticism made of Burns's work. Redwood survived with 0.62 percent and piñon pine with 6.3 percent of total solar radiation. Their curves showing the amount of dry weight produced at different light intensities show a straight line for redwood, which has the lowest light requirement, and curves convex downward for western yellow pine, Douglas fir, and piñon pine. The curve for Engelmann spruce is S-shaped.

Davis and Hoagland (8) reported on the growth of wheat under artificial conditions. They found the dry weight produced to be directly proportional to the light intensity over the range of intensities they used. Dry weight seemed to bear an exponential relation to the length of day.

### Effects of Light Quality on Growth

The effects of light quality upon the growth and form of plants was studied by Popp (22). He found very little difference between plants grown under full sunlight conditions and those grown in the absence of ultra-violet radiation. When all wave lengths shorter than 529  $m\mu$  were removed the growth was poor, the plants were weak, and lower in fresh and dry weight. When the dry weight per unit intensity is considered the plants grown under the complete solar spectrum appear to have the advantage. This result is exaggerated by the fact that the intensity was lower in this light quality than in any other except that of house 5, which eliminates all the blue. Table I is taken from Popp's data.

The effects of different ranges of wave lengths on the anatomical development of plants were studied by Miss Pfeiffer (20). The full solar spectrum gave better development as expressed by greater stem thickness, height, leaf thickness and differentiation, and extent of root system, than any of the other qualities used, with the visible spectrum next best. The red and blue were poorest, partially due to their lower intensities.

Sayre (25) studied the chlorophyll development in different spectral regions. He found no chlorophyll development in wave lengths longer than 680 millimicrons. In the visible region chlorophyll formation occurred in all spectral regions if sufficient energy were present, the red being most effective, followed by the green and blue. The chlorophyll development was judged by noting the relative greenness of the plants.

## METHODS

## Light Conditions

In experiments designed to study the effects of light conditions upon plant growth either sunlight or artificial light may be used. Sunlight varies over wide limits from day to day and from hour to hour, in addition to its seasonal variations. Davis and Hoagland (7) grew wheat plants for 30 days at different times during the year and found variations in dry weight of from 15.2 grams to 97.5 grams per 100 tops. The temperature was maintained at 20.5° C. throughout the year. These results are attributed to variations in solar radiation. The magnitude of the daily and seasonal variation in solar radiation may be appreciated from an examination of meteorological reports. All light experiments described below were carried out at the Boyce Thompson Institute for Plant Research at Yonkers, New York. The sunlight conditions prevailing during the time of any experiment are shown in the reports of the New York Meteorological Observatory.

Owing to these great variations in sunlight, it is difficult to carry out quantitative experiments on the effects of sunlight intensity on plant growth, and it is impossible ever to duplicate exactly the light conditions of one experiment in a later one. Artificial light may be maintained fairly constant, but it differs greatly in quality from sunlight. In this work both artificial light and sunlight have been used.

## Constant-condition Room

The plants grown under artificial light were placed in the constant-condition room at the Boyce Thompson Institute (3). In order to get a range of intensities two 1,500-watt lamps were suspended in front of the air inlet (Pl. XXVIII, fig. 1). The light intensities varied from 20 to 700 foot-candles depending upon the position in the room. Hourly variations in light intensity were negligible as shown by the pyrheliometric record.

TABLE 2. *Shading Cloth Used for Greenhouse and Out-of-door Shades*

Greenhouse Shade	Out-side Shade	Shading Cloth Used	Mesh (No. Threads per Inch)	Average Light Intensity (Percent of Outside)	
				Greenhouse	Outside
<i>E</i>	—	2 layers muslin	52 x 56	1.0	
<i>D</i>	<i>d</i>	1 layer muslin	52 x 56	8	20
<i>C</i>	<i>c</i>	1 layer cheesecloth	36 x 40	19	47
<i>B</i>	<i>b</i>	1 layer cheesecloth	24 x 24	40	74
<i>A</i>	<i>a</i>	none	—	71	100

The lamps did deteriorate during the course of the experiment and were replaced on May 1. Temperatures were uniform throughout the room. At a distance of two feet below the lamps the rise in temperature due to

their heat was less than  $0.5^{\circ}$  C. Continuous records of temperature as measured by both wet and dry bulb thermometers were taken by Cambridge resistance thermometers. The temperature varied from  $25^{\circ}$  to  $27^{\circ}$  C. with an average of  $26^{\circ}$  C. and the relative humidity varied from 89 to 93 percent in both light and dark rooms. Normal atmospheric carbon dioxide concentration was used.

The plants shaded one another to some extent. This shading was taken into account in making the light measurements.

### Greenhouse Shades

Four wooden frames,  $3 \times 5\frac{1}{2} \times 4$  feet high, were placed in the greenhouse and covered with different weaves of cloth to provide a range of light intensities (Pl. XXVIII, fig. 2). Table 2 shows the arrangement.

The transmission of the shading cloth was measured at 8 different wave lengths distributed over the visible spectrum by means of a König-Martens spectrophotometer. The transmission of the cloths used was the same for all wave lengths tested.

Measurements of light intensity in the different shades were taken with the Macbeth illuminometer on cloudless days between the hours of 11:00 A.M. and 12:30 P.M. The measurements were taken in foot-candles and compared to total daylight intensity. These percentage intensities

TABLE 3. *Temperature and Humidity Conditions in Greenhouse Shades, October 24-March 23, 1927-28*

Shade.....	E	D	C	B	A
Temperature, degrees C.					
Maximum recorded.....	28	28	29	30	30
Minimum recorded.....	19	19	19	19	19
Mean.....	24.2	24.0	23.9	23.3	23.7
Average algebraic deviation from daily mean.....	+0.5	+0.3	+0.2	-0.6	-0.4
Average absolute deviation from daily mean.....	0.6	0.4	0.4	0.8	0.9
Mean weekly evaporation from Livingston standardized atmometers, cc. of water.....	118	95	100	76	73

showed some variations with the progress of the season, due in part to the change in the angle with which the sun's rays struck the greenhouse glass. Owing to the seasonal variations in illumination only plants grown at the same time can be safely compared.

Each shade was provided with forced ventilation from a commercial air-conditioning machine. The temperature and humidity variations in the different shades were thus kept at a minimum (table 3).

Temperature conditions during the summer were higher and showed average deviations of  $0.5^{\circ}$  to  $0.9^{\circ}$  C.

The temperature table was made up from daily readings of thermometers placed in the different shades. Readings were made at all hours of the day, but most of them were made at about noon when the deviations were largest.

### Outside Shades

Three cubical wooden frames 8 feet on a side were set up in the garden and covered with one layer of the three shading cloths used in the greenhouse shades. Figure 3 of Plate XXVIII shows a view of a shade with one side removed. Forced ventilation was supplied the three shades from a fan at a rate calculated to provide for a complete air change every one and one-half minutes.

Temperature and evaporation conditions are shown in table 4.

TABLE 4. *Temperature and Evaporation Conditions in Outside Cages, June 8–August 1*

Shade .....	D	C	B	A
Light intensity, percent.....	20	47	74	100
Temperature, degrees C.				
Maximum recorded.....	32	34	34	33
Minimum recorded.....	15	14	14	14
Mean.....	25.2	25.0	24.7	24.8
Average algebraic deviation from daily mean....	+0.3	+0.1	-0.1	-0.3
Average absolute deviation from daily mean....	0.5	0.4	0.3	0.5
Mean weekly evaporation from Livingston standardized atmometer cups.....	47	66	72	85

### Spectral Houses

To test the effect of light quality upon growth, plants were grown in the spectral glass houses at the Institute. These houses were described by Popp (22). A blue glass has been substituted for the window glass and Corex has been substituted for *G 86 B*. The house numbers have been rearranged slightly.

Text-figure 1 shows the spectral transmission curves in the visible region of the different glasses used as measured on the König-Martens spectrophotometer. Text figure 2 and table 5 show the limits of transmission as determined by the Hilgar quartz spectrograph.

TABLE 5. *Visible and Ultra-violet Spectral Regions Transmitted by the Various Light Filters Used*

House No.	Name of Glass	Wave Lengths Transmitted (millimicrons)
I.....	Noviol "O"	389-720
II.....	"Corex"	290-720
III.....	G 403 ED	374-585
IV.....	Noviol "C"	472-720
V.....	G 34	529-720

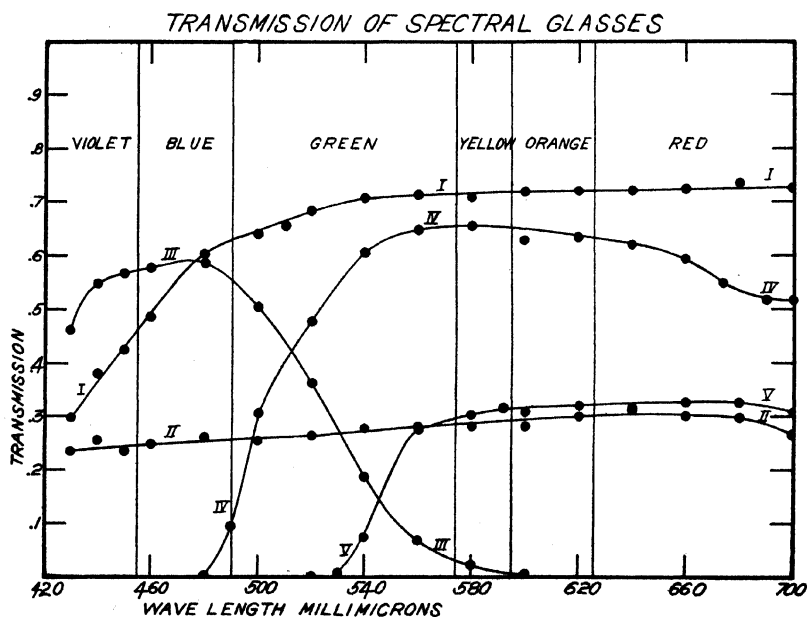
The glass was manufactured by the Corning Glass Company.



Temperature and humidity conditions prevailing in the houses at the time of growth are shown in table 6.

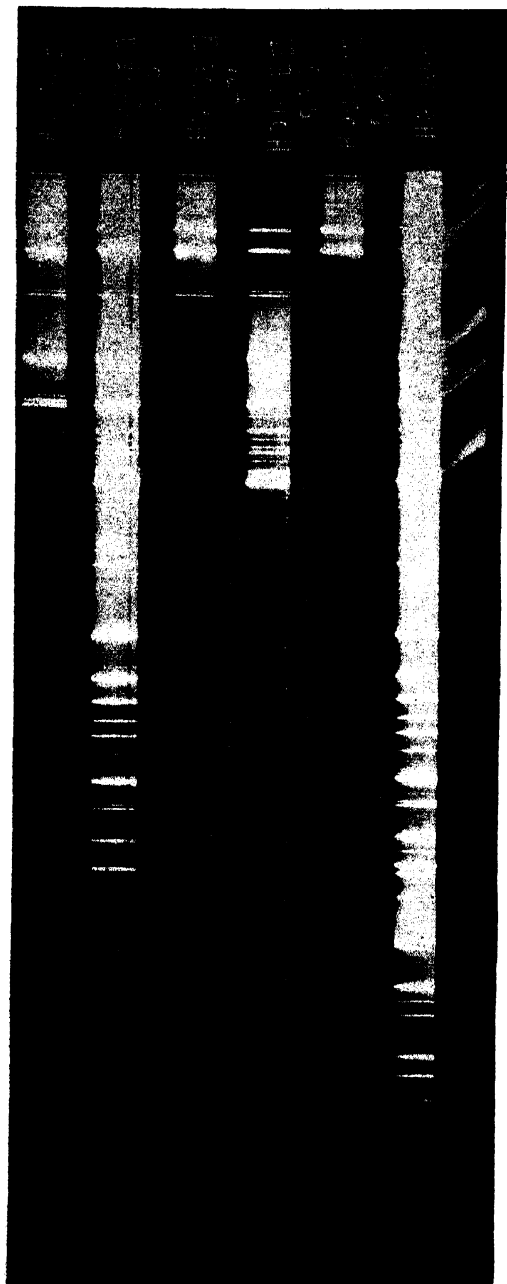
TABLE 6. *Temperature and Evaporation Conditions in Spectral Houses, July 11–October 22*

House. ....	I	II	III	IV	V
Temperature, degrees C.					
Maximum recorded.....	42	40	40	38	39
Minimum recorded.....	20	20	19	19	19
Mean.....	29.8	27.8	27.4	27.3	27.2
Average algebraic deviation from daily mean.....	+2.0	-0.1	-0.3	-0.6	-0.6
Average absolute deviation from daily mean.....	2.0	0.5	0.6	0.7	0.7
Mean weekly evaporation from Livingston standardized atmometers, cc. of water.....	104	143	130	143	122



TEXT FIG. 1. Transmission curves for glass of spectral houses. The Corex, house II, was unpolished. Actually it transmits about 80 percent of the light instead of 25 percent. The measurements were made on a König-Martens spectrophotometer.

In order to secure comparable light intensities two shades were arranged in each house, so that in each quality condition three intensity conditions were maintained. Light intensity measurements were made with thermopiles and with the Macbeth illuminometer. Measurements made with the two instruments showed fairly close agreement.



TEXT FIG. 2. Transmission spectra of the glasses used. Spectra obtained by use of a Hilgar quartz spectrograph illuminated with a mercury-vapor arc in quartz. The figures on the scale show the wave length in hundredths of microns.

## Effects of Differences in Soil Moisture on the Dry Weight and Root Development of the Bean Plant

Since it is very difficult to maintain soil moisture uniform in pots placed in a series of shades, the question arises as to whether or not slight variations in moisture are likely to cause significant differences in the dry weight. Accordingly a special experiment was carried out at the Connecticut Agricultural Experiment Station in New Haven, to answer this question.

An early variety of red bunch bean was grown in a rich medium sand soil in two-gallon glazed jars. The moisture content of the jars was controlled by filling to weight every other day. The water was added through a glass U-tube which ended in an inverted clay pot filled with gravel. The water distributed itself uniformly through the soil except for the upper inch in the drier pots.

The soil moisture varied from a point where the particles of soil would scarcely cohere, to a point where moisture would drip from the soil. The plants in the driest soils sometimes

TABLE 7. *The Influence of Soil Moisture on the Dry Weight and Root Development of the Bean*

Soil Moisture (percent of dry weight)	Dry Weight of Tops, to Plants (grams)	Root Development
7.....	22.6	very extensive
10.....	22.3	very extensive
11.....	20.8	very good
12.....	23.7	very good
15.....	22.6	good
16.....	22.3	good
18.....	20.3	good
18.....	24.9	good
25.....	29.4	poor
27.....	24.1	poor

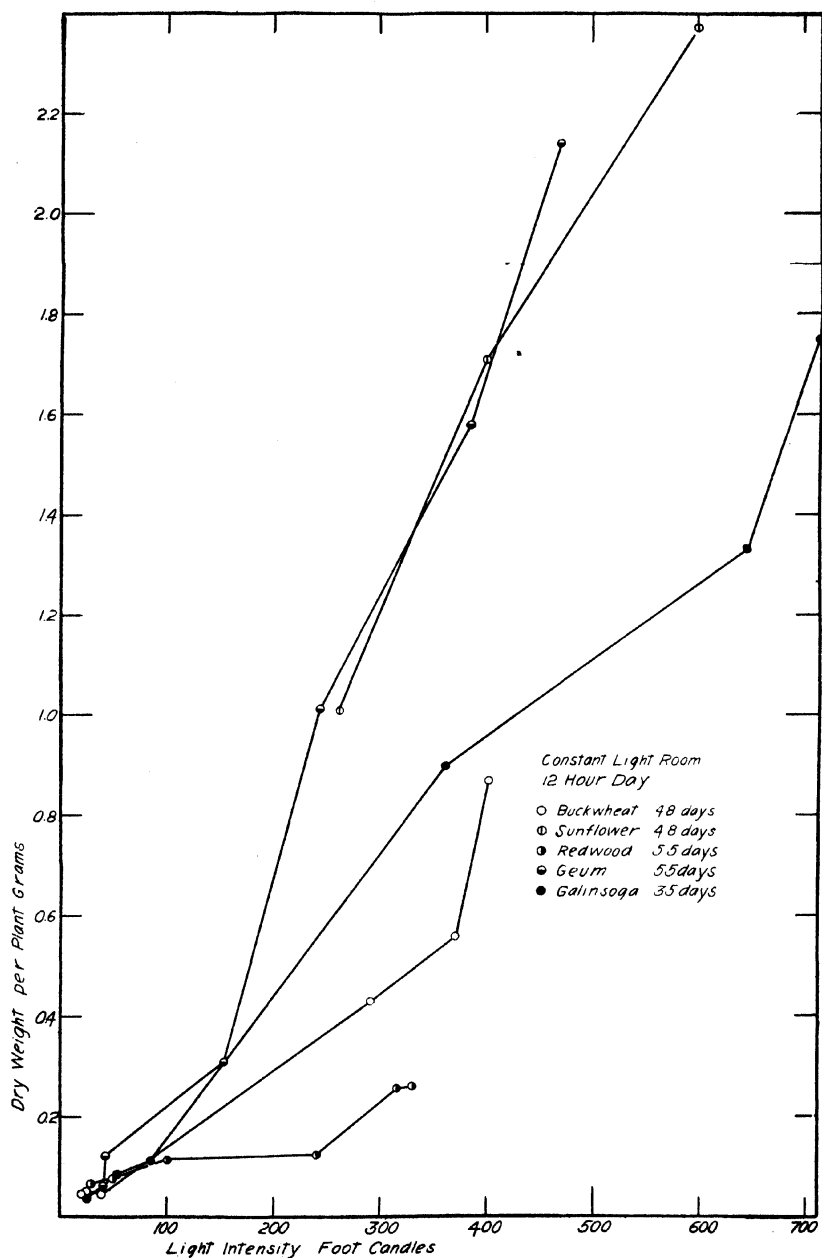
wilted slightly on bright days. Table 7 shows the results obtained. An examination of the table shows that no significant differences in dry weight were produced by the experimental conditions imposed on the plants.

### Species Used

The following species of plants were used in these investigations: buckwheat (*Fagopyrum esculentum* Moench.) variety Japanese; dwarf sunflower (*Helianthus cucumerifolius* Torr. and Gray); *Galinsoga parviflora* Cav.; avens (*Geum canadense* Jacq.); green and purple wandering Jew (*Tradescantia fluminensis* Vell. and *Zebrina pendula* Schnizl.); hog peanut (*Amphicarpa monoica* (L.) Ell.); California redwood (*Sequoia sempervirens* Endl.); loblolly pine (*Pinus Taeda* L.), tomato (*Lycopersicum esculentum* Mill.) variety Bonny Best; tobacco (*Nicotiana Tabacum* L.), variety Turkish.

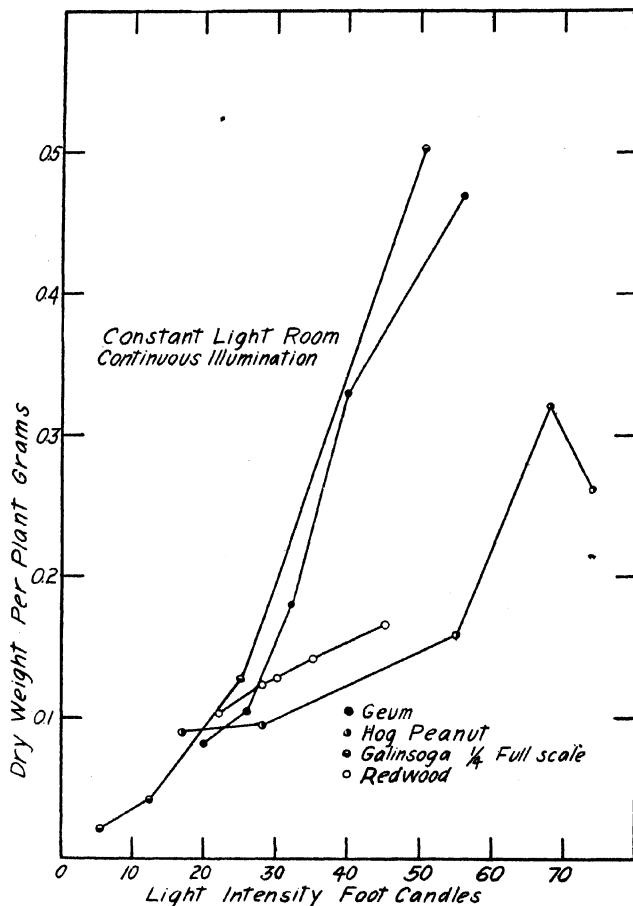
### Treatment of Plant Material Before and During the Experimental Period

The plants were grown in a rich composted loam soil, containing a high sand fraction. The soil was sterilized with steam and leached before using. Eight-inch clay pots were used for growing all plants except tomato and tobacco, which were grown in two-gallon jars perforated at the bottom. Buckwheat seed was sown in the pots. When the cotyledons had com-



TEXT FIG. 3. The influence of 12 hours daily artificial illumination of different intensities on the production of dry matter in plants. The dry weight is almost directly proportional to the light intensity.

pletely unfolded the plants were thinned to 15 to 25 plants per pot, and allowed two or three days to adjust themselves before placing under the shades. All other plants were transplanted to the pots soon after their first pair of leaves had unfolded. Redwood and loblolly pine seedlings were usually 2 to 5 centimeters high when placed in the cages. *Geum* was 1 to 2 months old. A sample of each species of plants was taken for dry-weight determinations at the start of an experiment to determine the



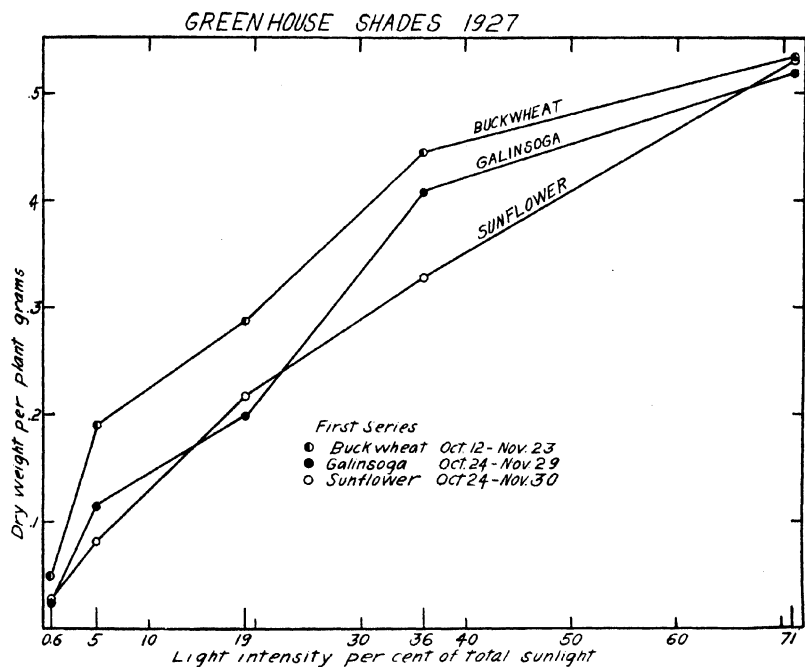
TEXT FIG. 4. The influence of continuous artificial illumination of different intensities on the production of dry matter in plants.

initial dry weight. For sunflower, buckwheat, and *Galinsoga* 15 to 20 plants were grown per pot. Loblolly pine was grown 10 to the pot, and *Geum* and redwood 5 plants to the pot. Tobacco and tomato were grown in individual pots.

The plants were watered and examined daily. Measurements were made of height each week, and notes taken on the general appearance and progress of the plants.

### Analysis of Plant Material

The annual plants were grown until flowering and usually until fruiting had started. Before harvesting, one or more average dominant plants were chosen from each pot for leaf area and chlorophyll determinations, except in tomato and tobacco where only one leaf from each plant was used. A blueprint of the leaves was made and the area determined by a planimeter. After blueprinting, the leaves were used for chlorophyll

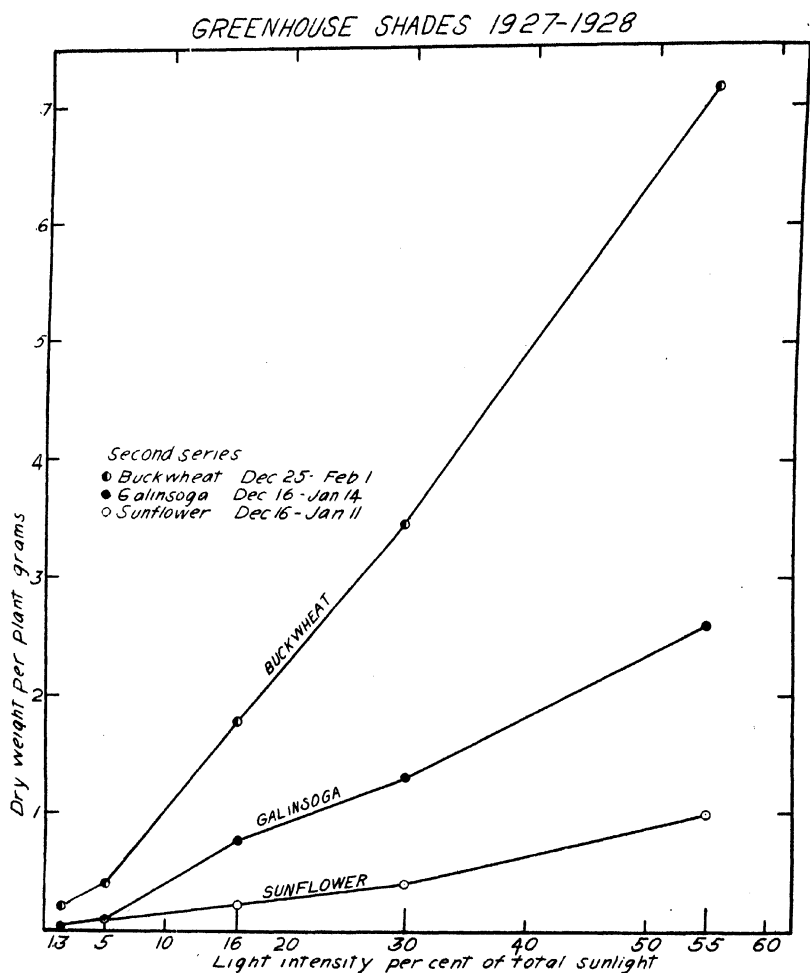


TEXT FIG. 5. The production of dry matter in different intensities of daylight. The curve for buckwheat is convex upwards while the others are approximately straight lines.

determinations. Chlorophyll was estimated by the method of Willstätter and Stoll as modified by Schertz (26). A colorimeter was used to compare the pigment with a standard made up from crystalline chlorophyll, and the standard described by Guthrie (12).

The remaining plants were used for fresh- and dry-weight determinations. The plants were cut off at the surface of the soil and the tops weighed

immediately. They were then taken to a cold room and frozen. The next day the frozen plants were run through a food chopper. The ground material was thoroughly mixed and a proportionate sample taken for



TEXT FIG. 6. The influence of shading in midwinter on the production of dry matter. These curves approximate straight lines closer than those of text figure 5 where the total amount of light received was greater.

moisture determination. If the plants were small the entire plant was used for a moisture sample. The moisture samples were dried to constant weight in a vacuum oven run at 70° C. The roots of redwood, loblolly pine, and *Geum* were washed out and dried separated from the tops.

In each experiment from 2 to 6 pots of plants were used in each light condition. In the analysis of the material for fresh and dry weight the plants of each pot were treated separately so that the average deviations for the individual pots could be calculated. The complete data taken on each set of plants were considered too bulky to be included in this paper. The curves and data presented are the mean values from a large number of plants. In the constant-condition room 20 redwood and *Geum* plants were grown in each intensity and 40 of all other plants. In the spectral house shades 10 *Geum* plants and 30 sunflowers and *Galinsoga* plants were grown in each condition. In the greenhouse and outside shades from 15 to 30 of each perennial species, and 30 to 150 of each annual species, were used in each shade except tomato and tobacco, of which six were used.

## RESULTS

### Light Intensity Studies

In the constant-condition room with 12 hours daily illumination all plants except sunflower survived for the period grown with less than 50 foot-candles illumination (table 8). Although sunflower showed some

TABLE 8. *Survival at Low Intensities*

Plant	No. of Days Grown	Light Intensity (foot-candles)	Percentage Survival	Percentage Increase in Dry Weight
Sunflower.....	48	46	0	—
<i>Geum</i> .....	54	41	90	358
Redwood.....	55	30	90	66
<i>Galinsoga</i> .....	35	25	92	1,670
Buckwheat.....	48	26	96	2,500

growth at first in the lower intensities, it died before the experiment was closed. The buckwheat plants had started to die in the lower intensities when harvested.

Of the plants grown under shades, all except sunflower showed 50-percent survival under the lowest intensities maintained, from 0.5 to 1.5 percent of total sunlight. Sunflower survived better under 1-percent intensity during the winter than during the summer. This was probably due to the higher temperature in summer which increased the rate of respiration. Redwood plants survived for 6 months with 1-percent sunlight intensity but half of them had died by the end of the seventh month. Loblolly pine survived five months under the same conditions but was dying by the end of the sixth. The herbaceous plants were grown for much shorter periods of time.

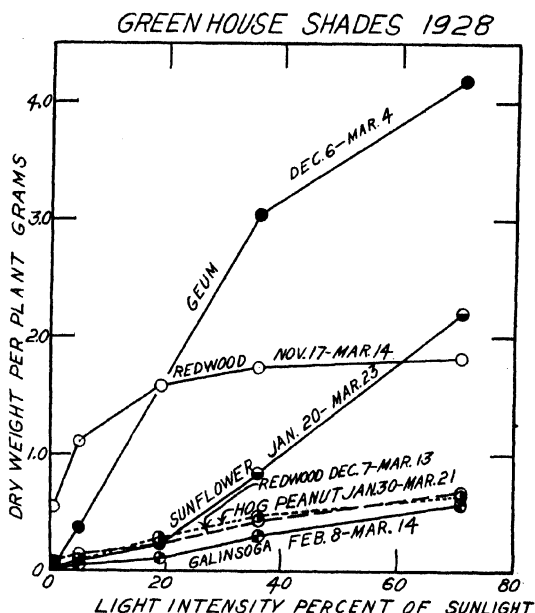
### *Production of Dry Matter*

With twelve hours daily illumination in the constant-condition rooms the dry weight produced by the plants grown was almost directly pro-



portional to the light intensity up to the highest intensities available under the two 1,500-watt lamps. A similar result was obtained with continuous illumination except that at 70 foot-candles hog peanut showed a decrease due to the injury caused by continuous illumination (text figs. 3 and 4).

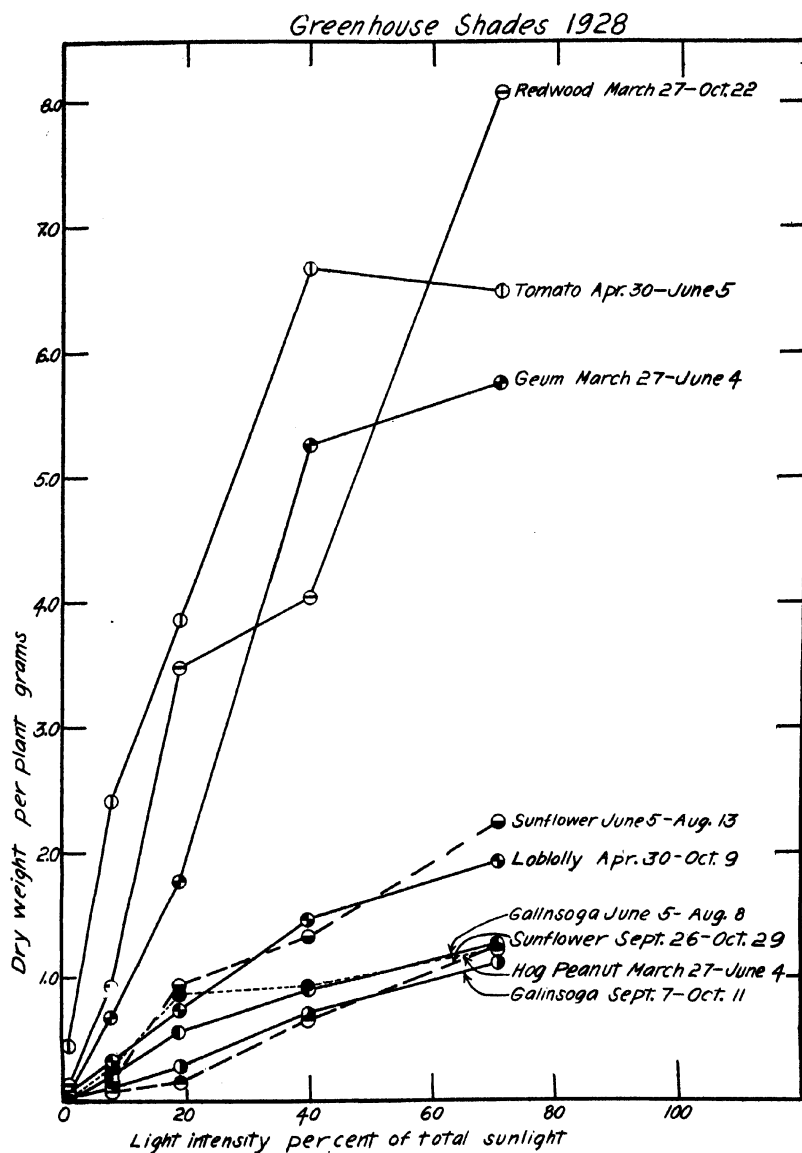
During the winter the dry weight produced by the shaded plants was almost directly proportional to the light intensity received up to the highest intensities available in the greenhouse (text figs. 5, 6, 7). During the summer, some plants in the greenhouse shades showed a tendency to use the light less efficiently at the higher intensities (text fig. 8). The plants grown in the outside shades under lower night and day temperature conditions showed different shaped curves (text fig. 9). The slope of the



TEXT FIG. 7. The effect of different daylight intensities on the production of dry matter in plants in the early spring. Only one series of redwood and *Geum* gave curves convex upwards. The relative positions of the curves are not significant.

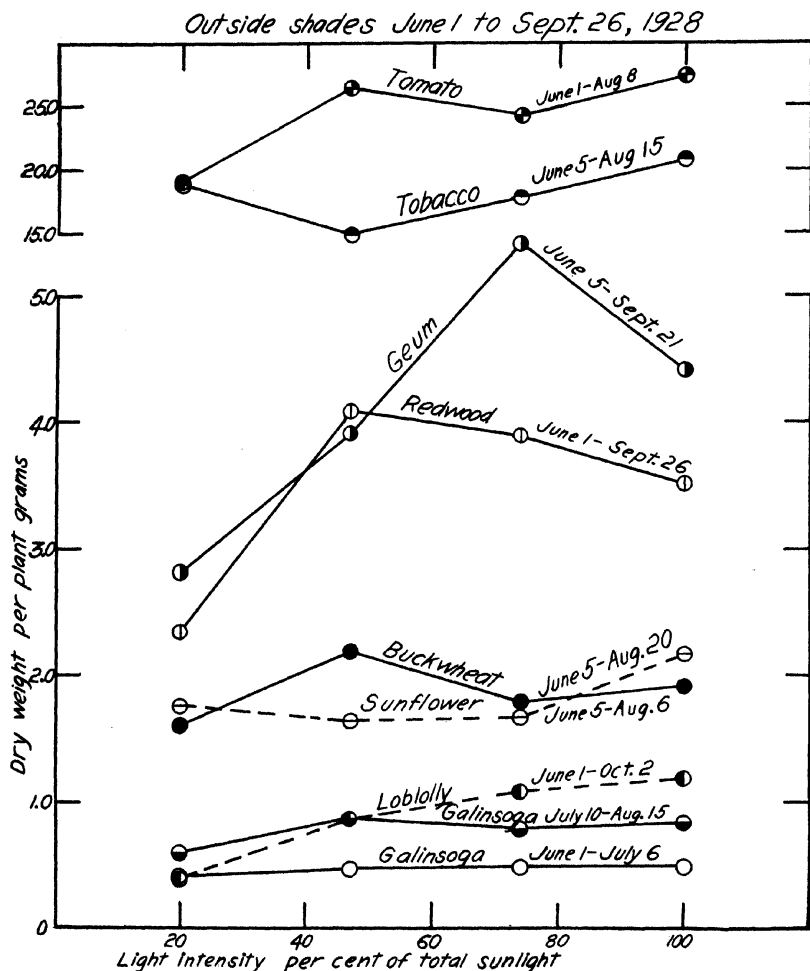
curves decreased and there was a tendency to develop maximum dry weight at lower light intensities than inside. The light intensity may be reduced 50 percent during midsummer without seriously affecting the growth of the plants studied. A further reduction to 20-percent intensity caused decreased dry weight for most of the plants used. Later in the season the same plants gave curves convex downward but not tending to run parallel to the axis (text fig. 10).

Redwood and *Geum* produced maximum dry weight at 50 and 75



TEXT FIG. 8. The effect of different degrees of shading on the production of dry matter in plants grown in the greenhouse in midsummer. Tomato, hog peanut, and *Geum* show a decrease in the efficiency with which they use light at the higher intensities. For all other plants the dry weight is almost directly proportional to the light intensity. The relative position of these curves is not significant.

percent of full summer sunlight. When placed in direct sunlight, growth in these plants, together with loblolly pine, appeared to have been arrested. They immediately developed considerable red pigment in the leaves, while the tips of the leaves turned brown and died. Later they apparently

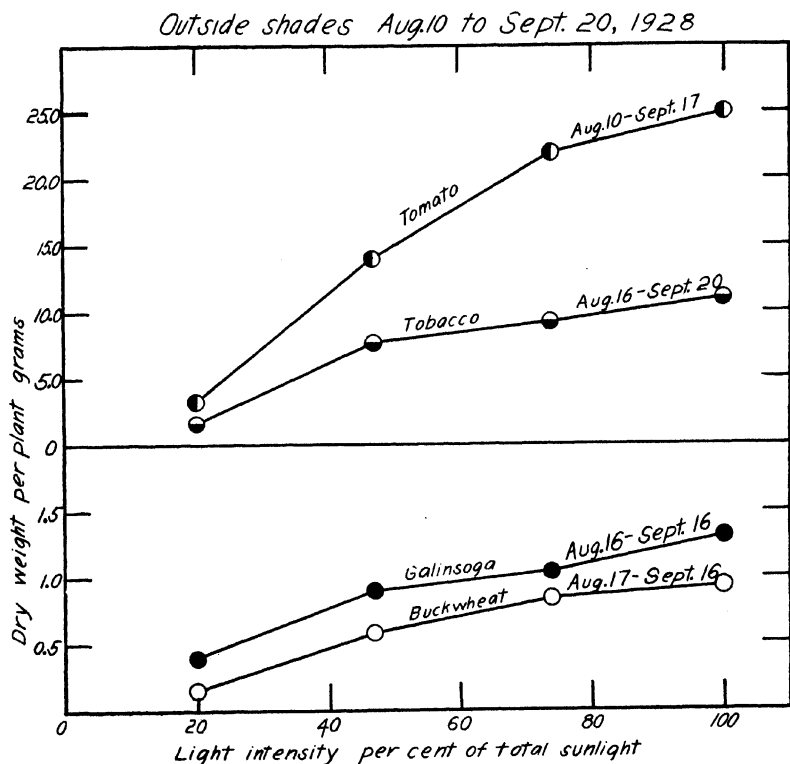


TEXT FIG. 9. The effect of different daylight intensities on the production of dry matter in plants grown out-of-doors in midsummer. At this time of year a reduction to one-half of full daylight caused little decrease in dry weight.

became adjusted and were growing nicely when harvested. All other plants used produced maximum dry weight in full summer sunlight.

A careful examination of the curves will show a slightly different shape

for different species under comparable light intensities. The curves for *Geum*, hog peanut, redwood, tomato, and buckwheat show a tendency towards being convex upwards while for sunflowers, tobacco, and *Galinsoga* the curves are nearer straight lines or convex downward. As one would expect, plants which usually grow in the shade, as *Geum* and hog peanut,



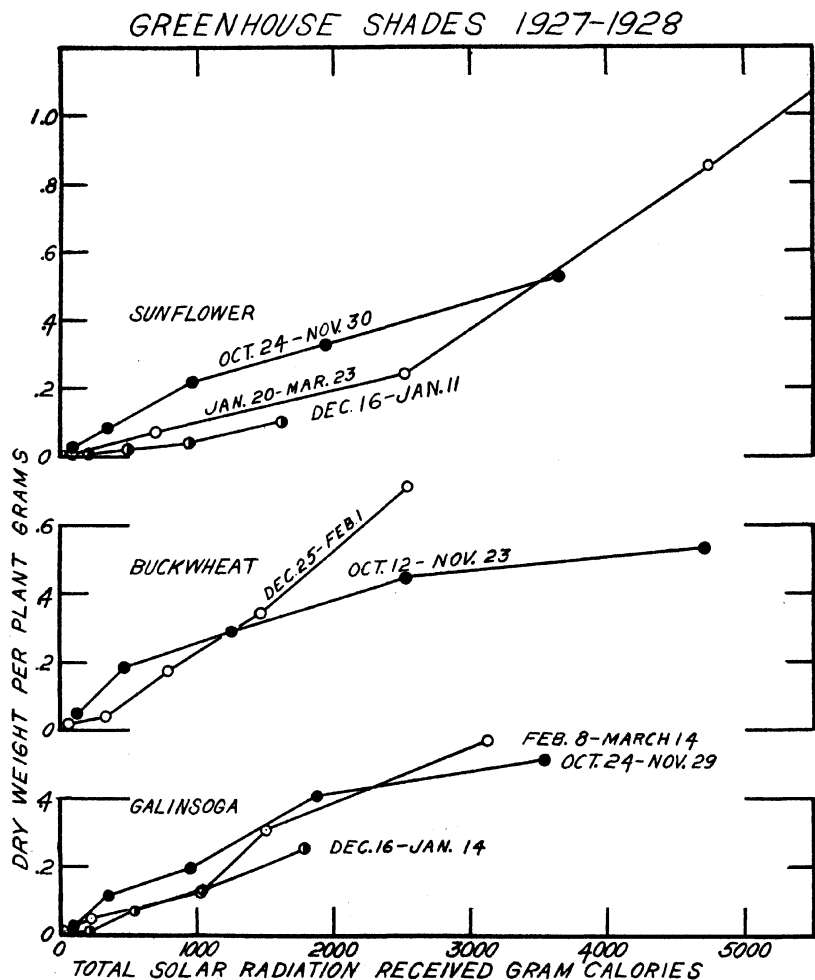
TEXT FIG. 10. The production of dry matter in plants under a range of daylight intensities in the late summer. Any decrease in intensity at this time of year caused a corresponding decrease in the production of dry matter.

were found to use light less efficiently at high intensities than at low ones. On the other hand, one would expect plants which usually grow in sunny habitats to use light with equal or greater efficiency at moderately high intensities than at low intensities. Sunflower and *Galinsoga* maintained their initial efficiency rate at considerably higher intensities than *Geum* and hog peanut.

#### *Total Solar Radiation Required for Growth*

Hourly records of the total solar radiation in gram calories received on a square centimeter of horizontal surface are published each month by the

New York Meteorological observatory located in Central Park, New York City. The Boyce Thompson Institute for Plant Research is only 13 miles from this observatory, so it seems reasonable to assume that the radiation

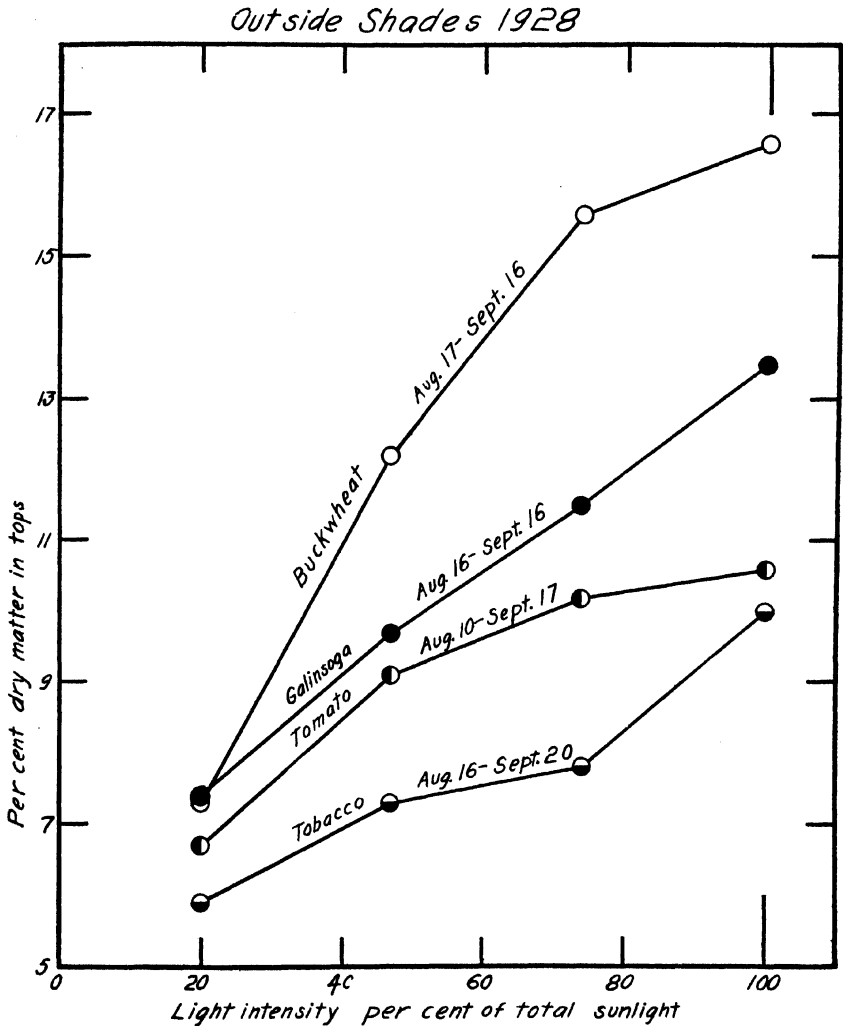


TEXT FIG. 11. The influence of total solar radiation received on the production of dry matter in plants. Note that the plants receiving the longer daily period of illumination grew better than those grown in midwinter.

received at the Institute over a period of a month or more would be approximately equal to that received at Central Park.

Text figure 11 shows the amount of dry weight produced per plant per gram calory of radiation received on a square centimeter of horizontal

surface. During the period over which these plants were grown the temperatures were held fairly uniform as shown above. It will be noticed that for both *Galinsoga* and sunflower the plants grown in midwinter with



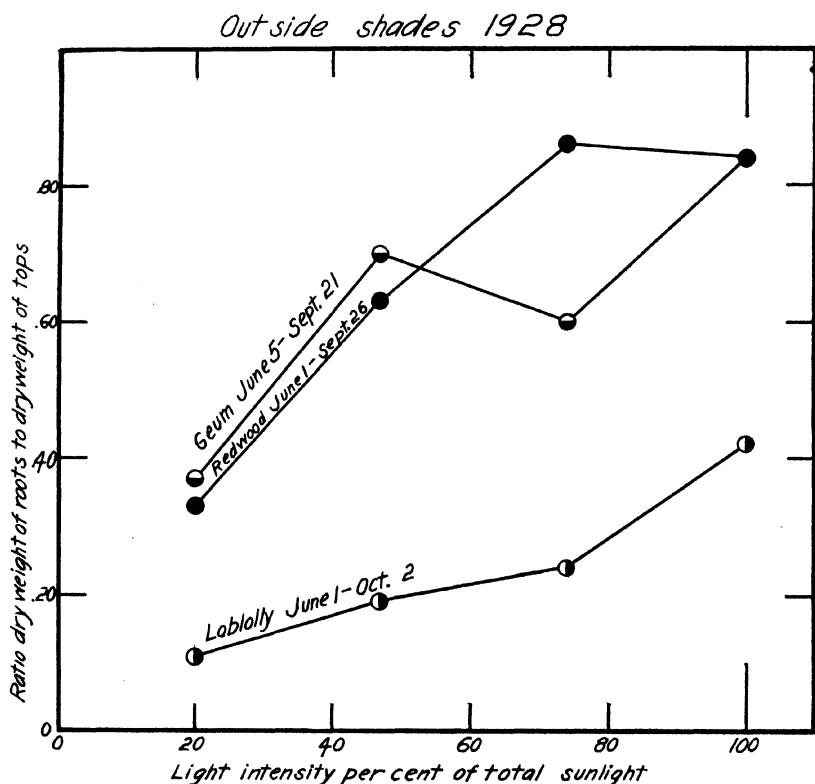
TEXT FIG. 12. The influence of light intensity upon the percentage of dry matter in plants.

the shortest day lengths produced the least dry matter per unit energy received. This is in accord with results presented by Davis and Hoagland (8). Aside from this point the correlation between plants grown at one

time as compared with another is not close. This lack of correlation is partly due to the fact that the plants were not all harvested at the same age.

### *Percentage of Dry Matter*

The percentage of dry matter in the tops of plants increased with increasing light intensity, as shown in text figure 12.



TEXT FIG. 13. The influence of light intensity on the proportion of root to shoot.

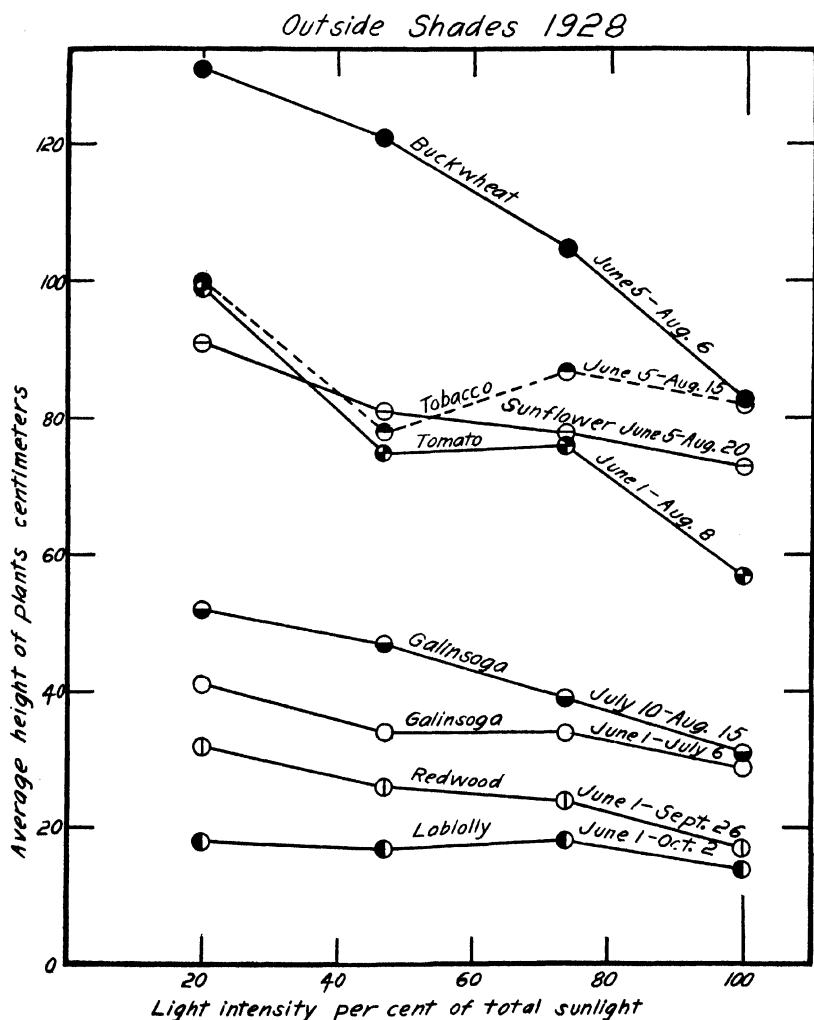
### *Ratio of Root to Shoot*

Low light intensity favors top growth at the expense of root growth. This is well demonstrated in the ratio of dry weight of roots to dry weight of tops (text fig. 13 and Pl. XXIX).

### *Height Growth*

With decreasing light intensity the plants studied tend to increase their height. The height attains a maximum at about 20 percent of full summer sunlight, or 60 percent of late summer sunlight (text figs. 14 and 15 and Pls. XXIX-XXXI). Upon further decrease in light intensity the height

falls off rapidly. It seems evident that the light intensity cannot be reduced below the point at which maximum height growth occurs without causing incipient starvation of the plant. Plants under high light intensities tend



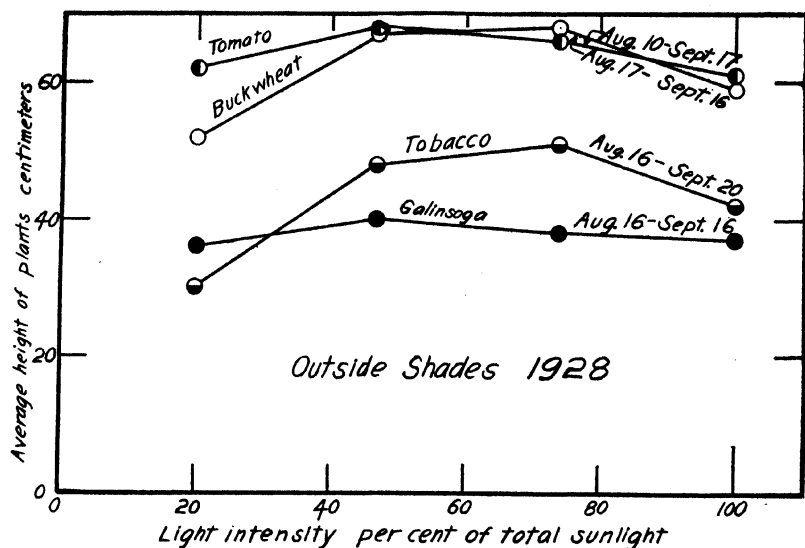
TEXT FIG. 14. The influence of light intensity on the height growth of plants. Contrast with text figure 9, which shows the dry weight of the same plants. The tallest plants usually had the least dry weight.

to attain complete height growth earlier than shaded plants due to the fact that they mature earlier (text fig. 16).



*Strength of Stem and Form of Plant*

The stems of the plants grown under low intensities were weak and succulent, often too weak to support the plant (Pls. XXIX-XXXI). The lack of sufficient woody material accounts for their weakness and brittleness. The heavily shaded plants had long internodes and few branches. The density of growth increased with increasing light intensity



TEXT FIG. 15. The influence of shading in late summer on the height growth of plants. At this season a decrease to 20-percent intensity caused a decrease in height growth. Text figure 10 shows the dry weights of these plants.

*Leaf Development*

The leaf area per plant seemed to follow height growth rather closely and attained a maximum at about the same light intensities. Leaf thickness increased with increasing light intensity as shown by the weight of 100 square centimeters of leaves and by cross section studies (text fig. 17). The internal structure was also modified by light intensities. Under low intensities the palisade tissue decreased from two layers to one layer, while the intercellular spaces increased slightly.

*Chlorophyll Concentration*

Table 9 shows the chlorophyll concentrations per unit leaf weight and per unit leaf area. For all plants studied there is a tendency to increase the chlorophyll concentration with decreasing light intensities until a critical intensity is reached. Further decrease in light intensity causes a decrease in chlorophyll concentration. This is more pronounced when

TABLE 9. *Chlorophyll Concentration in Leaves*

Plant Material. . . . .	Chlorophyll per 10 Grams Fresh Leaves (milligrams)					Chlorophyll per 100 Sq. Cms. Leaves (milligrams)				
Outside Shades		20	47	74	100		20	47	74	100
Light Intensity (percent). . . . .										
Sunflowers, June 5-Aug. 20. . . . .		17	11	11	9		3.1	2.3	2.3	2.7
Tomato, June 1-Aug. 8. . . . .		20	15	14	12		3.5	3.3	3.2	3.6
Tobacco, June 5-Aug. 15. . . . .		14	10	11	9		2.7	2.9	3.4	2.7
<i>Galinsoga</i> , June 1-July 5. . . . .		18	11	11	12		2.7	1.8	1.9	2.3
<i>Geum</i> , June 5-Sept. 21. . . . .		16	16	10	12		3.5	4.7	3.3	3.7
<i>Galinsoga</i> , Aug. 16-Sept. 16. . . . .		32	20	24	18		5.1	2.8	3.7	2.9
Tomato, Aug. 10-Sept. 17. . . . .		26	15	15	10		3.5	3.0	3.2	2.9
Buckwheat, Aug. 17-Sept. 16. . . . .		19	23	19	16		2.9	3.4	2.8	2.5
Greenhouse Shades										
Light Intensity (percent). . . . .	I	8	19	40	71	I	8	19	40	71
Hog peanut, March 27-June 4. . . . .	55	63	41	38	28	3.1	1.9	1.3	1.3	1.2
<i>Geum</i> , March 27-June 4. . . . .	28	34	33	27	24	3.6	4.1	4.6	4.7	4.0
<i>Galinsoga</i> , June 5-Aug. 8. . . . .	21	29	31	25	22	3.3	3.1	3.4	3.0	3.0
Tomato, April 30-June 5. . . . .	9	22	18	13	11	1.9	2.8	3.1	3.1	2.4
Sunflower, June 5-Aug. 13. . . . .	—	17	17	20	12	—	2.5	2.8	3.3	2.3

considered on the basis of leaf weight than on the basis of leaf area. Sunflower has a relatively low pigment concentration, and shows relatively slight variations with light intensity. Hog peanut, on the other hand, showed higher concentrations and larger variations under different intensities than the other plants used.

#### *Flowering and Fruiting*

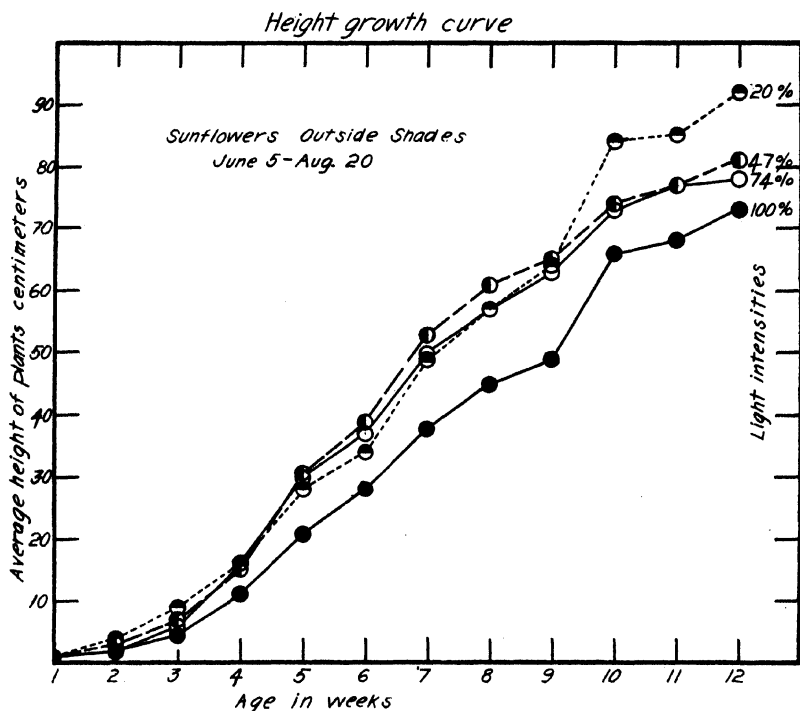
The first flowers were produced at about the same time under all light intensities unless the light intensity was so low as to seriously stunt the growth. While shading caused no appreciable delay in the time of appearance of the first flowers, maximum flower development occurred earlier with the plants receiving the higher intensities. Ripened fruit also could be found in all intensities at about the same time; however, in the higher intensities maximum fruit development was earlier. Shading seemed to prolong the vegetative and fruiting periods, while full sunlight intensities hastened maturity. These observations on flowering and fruiting are based on buckwheat and *Galinsoga* which flower and fruit on any day length from 9 hours to 24 hours. Plants grown with less than 10 percent of full summer sunlight produced only occasional flowers and no ripened fruit. Sunflower never flowered on less than 19-percent intensity; buckwheat and *Galinsoga*, on the other hand, flowered with 8-percent intensity but did not fruit.

#### **Light Quality Studies**

##### *Dry Weight*

Table 10 shows the production of dry matter with 10-percent light intensity in the different spectral regions. The dry weight curves for *Geum* are shown in text figure 18.

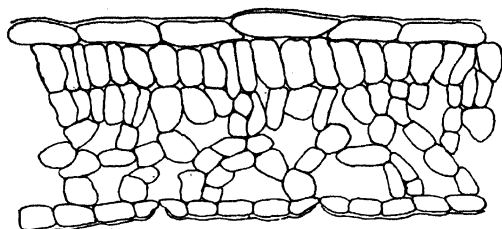
The entire visible and ultra-violet solar spectrum, house II, is more efficient for the production of dry matter in the plants grown than any of the other qualities used. Removal of the ultra-violet and some violet, house I, causes no very significant decrease in efficiency. Blue light, house III, also gave satisfactory growth. When all the blue is removed, as in



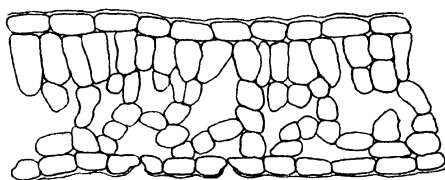
TEXT FIG. 16. The influence of different daylight intensities on the weekly height growth of sunflowers. The plants in full daylight were always shorter. Those receiving 20-percent intensity continued rapid height growth after the others had begun to mature.

TABLE 10. *Spectral Shades. Dry Weight per Plant in Grams Under 10-Percent Light Intensity*

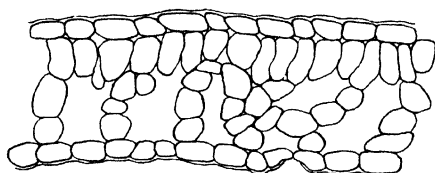
	Wave Lengths Transmitted (millimicrons)				
	II 290-720	III 374-585	I 389-720	IV 472-720	V 529-720
<i>Galinsoga</i> , May 30-July 5.....	.57	.31	.40	.30	.26
<i>Galinsoga</i> , July 1-Aug. 15.....	.28	.20	.29	.16	.10
<i>Galinsoga</i> , Sept. 9-Oct. 19.....	.55	.27	.63	.37	.17
Sunflower, June 5-Aug. 13.....	1.16	.55	.49	.13	.04
Sunflower, Sept. 19-Nov. 1.....	.40	.22	.55	.20	.11
<i>Geum</i> , July 10-Oct. 24.....	2.30	1.32	1.08	1.26	.63



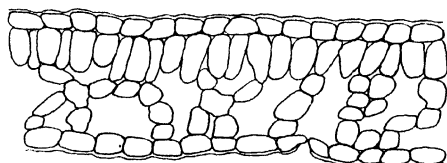
BED 1



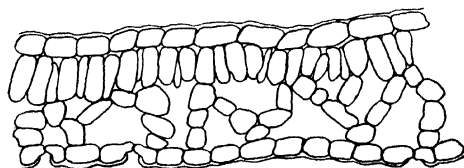
BED 2



BED 3



BED 4



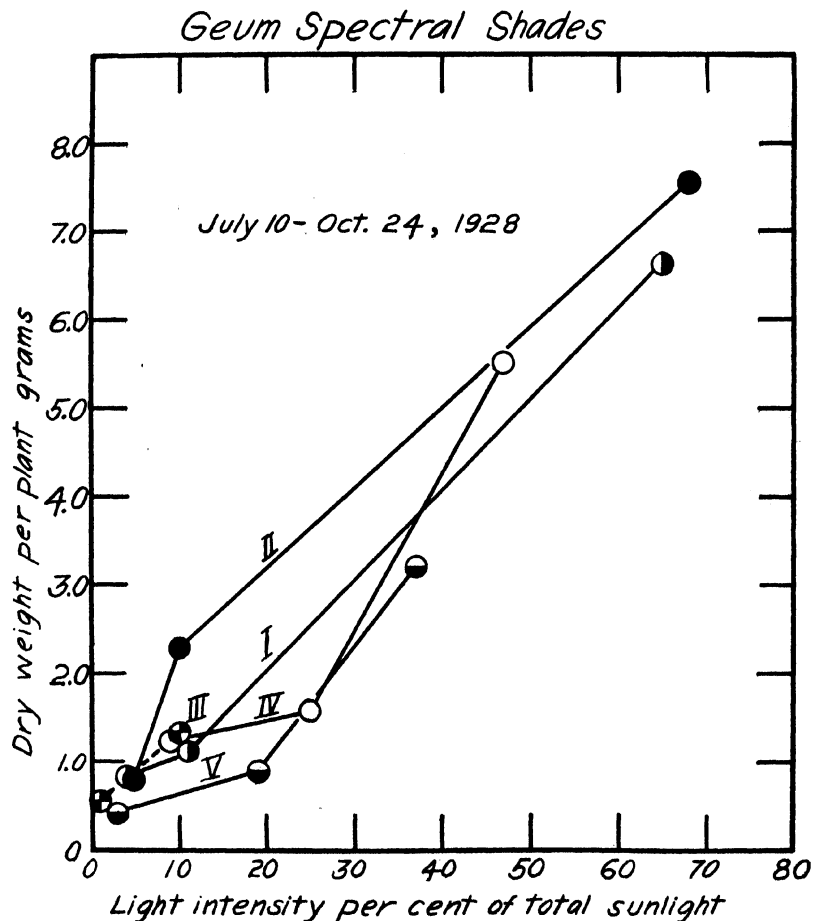
BED 5

TEXT FIG. 17. Camera lucida drawings of cross sections of buckwheat leaves of Oct. 12 to Nov. 23, 1927 series. The light intensities of beds 1 to 5 were respectively 71, 36, 19, 5, and 0.6 percent of full daylight. The lower intensities cause a decrease in the number of palisade layers, in the size of cells, and in total leaf thickness. The intercellular spaces increased somewhat.

house V, a serious decrease in efficiency results, while house IV is intermediate in efficiency.

*Form and Vigor*

Normal plants have not been grown without the use of blue light (see Pl. XXXII). The plants of house V, minus blue, were tall and weak, thus suggesting etiolated plants in appearance. The leaves were badly rolled and often crinkled. Plants grown under the blue glass seemed to be stunted somewhat, having very short internodes but rather sturdy stems. The



TEXT FIG. 18. The effect of different light qualities and intensities on the production of dry matter in *Geum*. Curve II is for normal daylight; curve I is minus ultra-violet; curve III (dotted) is blue light; curve IV is minus violet and part of blue; curve V is minus all the blue. Quality II is most efficient and quality V is the least efficient.

plants from house II were always most sturdy and vigorous, followed by those of houses I and IV. Blue light if of sufficient intensity prevents excessive elongation and an etiolated type of growth.

### *Chlorophyll Concentration*

Table II shows the chlorophyll concentrations in the different houses.

TABLE II. *Chlorophyll Concentration, Spectral House Shades*

Spectral Region Used (wave length mμ)	Light Intensity (percent)	Chlorophyll per 10 Gms. Fresh Leaves (mgs.)					Chlorophyll per 100 Sq. Cms. Leaves (mgs.)				
		<i>Grum.</i> July 10-Oct. 24	Sunflower, June 5-Aug. 13	Sunflower, Sept. 19-Nov. 1	<i>Galinsoga</i> , May 30-July 5	<i>Galinsoga</i> , Sept. 7-Oct. 19	<i>Grum.</i> July 10-Oct. 24	Sunflower, June 5-Aug. 13	Sunflower, Sept. 19-Nov. 1	<i>Galinsoga</i> , May 30-July 5	<i>Galinsoga</i> , Sept. 7-Oct. 19
389-720	65	15	12	17	14	15	3.7	2.0	5.8	2.1	2.2
	11	25	17	23	26	31	4.8	2.7	4.1	3.3	3.7
	4	25	—	20	27	36	3.5	—	3.1	4.0	5.7
290-720	68	12	15	18	13	15	2.8	3.2	2.6	2.2	2.3
	10	29	16	27	28	38	5.5	2.8	4.6	3.8	4.0
	5	27	—	22	29	31	3.7	—	3.6	3.6	3.7
374-585	10	20	16	49	25	29	3.9	3.4	4.0	3.6	4.0
	4	25	—	25	26	23	3.3	—	5.2	3.7	3.8
	1	49	—	20	31	28	7.3	—	4.2	4.5	4.7
472-720	47	13	14	20	17	18	2.7	3.1	3.9	2.3	2.2
	25	23	18	27	29	24	3.6	2.7	4.4	3.8	3.2
	9	18	—	25	29	29	2.8	—	4.0	3.5	4.1
529-720	37	16	20	12	18	19	2.8	4.3	5.3	2.7	3.0
	19	17	—	17	24	11	3.3	—	4.1	3.7	3.6
	3	24	—	17	23	20	3.9	—	2.4	4.1	3.4

In all light qualities used, the plants increased their chlorophyll concentration with decreasing intensity to a certain point. For 10-percent intensity all qualities gave approximately the same chlorophyll concentration—house V was usually lower and house III often higher than the others.

### DISCUSSION OF RESULTS

The light intensities needed for *survival* of the plants used seem to be even much lower than the values given for other plants by Bates and Roeser (1), Burns (5), and Grasovsky (11). However, the determination of the minimum light intensity required for mere existence of a particular species is probably of little ecological significance, since the plants studied demand ten times as much light or more for flowering and fruiting. It seems probable that if several species of plants were competing on an area which was illuminated by a light intensity well above that required for

survival the plants having the fastest growth rate would become dominant, at least for the first generation, rather than those having the lowest light requirement for survival. The difference in the ability to survive under low light intensities may be due not so much to differences in the efficiency of the photosynthetic equipment of different species as to differences in the basal metabolism of the growing plant.

With increasing light intensity the rate of growth as measured by increase in dry matter is almost directly proportional to the light intensity up to 20 to 30 percent of full summer sunlight. Above 50-percent intensity the amount of growth increases very little with further increases in light. While slight shading causes no marked deleterious effects, shading which cuts out 80 percent or more of the light reduces the amount of growth considerably.

The optimum light intensities for the production of dry matter were much higher than those found by Grasovsky (11), Lubimenko (19), and Shantz (27). They are in approximate accord with the values given by Combes (6), Garner and Allard (10), Popp (21), Rosé (23), and Zillich (29). In view of the wide differences in species used, methods of experimentation, and especially of temperature and sunlight conditions, close agreement cannot be expected.

The differences in the dry weight curves for the plants grown in the greenhouse shades compared to the plants grown in the outside shades at the same time are attributed in part to the higher range of light intensity outside, but largely to the higher temperature conditions prevailing inside the greenhouse. Temperature must have acted as a partially limiting factor in the outside shades. This is in accord with the conclusions of Blackman and Matthaei (2) for the influence of temperature on the rate of photosynthesis, and with data presented by Davis and Hoagland (8), which showed that the temperature required for optimum dry weight production increased with increasing light intensity, within certain limits.

The correlation between dry weight produced and total solar energy received is not close. To secure good correlation of these factors the temperatures should be uniform, the day lengths should be the same, the plants should be harvested at the same age, and the light intensities should not be so high at any time as to either directly or indirectly inhibit the rate of photosynthesis.

A number of investigators have reported on experiments on the effects of light conditions upon the growth of plants in which height measurements and notes of general vigor were used as criteria of growth. For the plants used by the writer height and general appearance proved to be the least reliable criteria, while dry weight of the entire plant and dry weight of the fruit proved the most reliable. A comparison of text figures 9 and 10 with 14 and 15 shows that dry weights increased while height decreased with increasing light intensity. Even fresh weight may be deceptive due to

the much greater water content of the shaded plants. The necessity of considering the roots of woody plants is well emphasized by the redwood from the outside shades. At 20-percent intensity the root formed only 25 percent of the dry weight of the entire plant, while at 100-percent intensity the root formed 46 percent of the total dry weight. Low light intensities tend to produce vegetative growth at the expense of flowers and fruit, top growth at the expense of root growth, large leaf area at the expense of leaf thickness, and succulence at the expense of sturdiness.

Of the plants investigated sunflower is the most exacting. It needed more light for survival, more for flowering and fruiting, more for maximum height growth, and more for attaining maximum dry weight. Not only was sunflower more exacting in its intensity demands but it also showed much greater injury when grown in fractional parts of the solar spectrum than the other plants used. Sunflower was able to use the light with almost equal efficiency up to the highest intensities used, provided the temperature was sufficiently high, while *Geum* and hog peanut showed a decided decrease in efficiency at the higher intensities. The increased efficiency of *Geum* and hog peanut at lower intensities may be attributed in part to their ability to increase their chlorophyll concentration.

#### ECOLOGICAL SIGNIFICANCE

Since the light intensities under forest canopies are only 0.16 to 20 percent of full sunlight, and since a reduction of the light intensity below 20 percent of full sunlight causes a marked decrease in the amount of dry matter produced by plants, it seems evident that the light intensities prevailing under well stocked stands of forest trees are almost always below the optimum for the growth of higher plants, and may often approach the limiting values for survival. However, it should be borne in mind that light is not the only growth factor which may be below the optimum in the forest.

The change in quality of the light passing through the forest canopy, on the other hand is not a serious factor in the growth of the plants. The loss of the blue spectral region due to absorption by the leaves is more than compensated for by the gain in percentage of skylight as shown by Klugh's measurements (15, 16). The loss in the red region causes no significant difference in the efficiency of the light as shown by studies in the spectral houses. It seems certain that the poor growth of plants under forest canopies is not to be attributed to changes in light quality.

The increase in the percentage of the blue spectral region of forest light compared to daylight will cause intensity determinations, made by the use of light-sensitive paper, which is affected more by the blue region than by the red region, to be too high. Only methods which are uniformly sensitive to all wave lengths can be safely used in comparing forest light with daylight.



## SUMMARY

1. Plants were grown under four sets of light conditions: (1) under cloth shades inside a greenhouse; (2) under cloth shades out-of-doors; (3) in a constant-condition room supplied with artificial illumination; (4) in a series of houses covered with glasses transmitting definite spectral regions. Curves are presented showing the influence of light intensity upon the total production of dry matter, percentage of dry matter, height growth, and ratio of roots to shoots. Studies of chlorophyll concentration, leaf area, and time of flowering and fruiting were also made. The effects of different qualities of light on growth are shown. A discussion of the silvical and ecological significance of the studies is given.

2. The light needed for the survival of the plants grown is very low, being less than 40 foot-candles for all except sunflower, which requires a much higher intensity.

3. Redwood and loblolly pine are able to survive for a period of 6 months under a light intensity at which they are barely able to increase in dry weight. Sunflowers, on the other hand, died within two or three weeks.

4. At low light intensities the dry weight produced by the plants studied is almost directly proportional to the intensity received up to about 20 percent of full summer sunlight. At higher intensities the slope of the curve falls off, shade plants showing a decrease at lower intensities than sun plants.

5. The percentage of dry matter in tops, the ratio of dry weight of roots to dry weight of shoots, the density of growth, the strength of stem, and the leaf thickness all increased with increasing light intensity.

6. Leaf area and height attained maxima at light intensities of about 20 percent of full summer sunlight.

7. Chlorophyll concentration increased with decreasing light intensity until the intensity was so low that it hazarded survival. Further decrease in light intensity caused a decrease in chlorophyll concentration.

8. The time of maximum flowering and fruiting was considerably delayed by low light intensities. Fruiting did not occur at all in the plants studied in intensities below 8 percent of full summer sunlight.

9. The entire visible and ultra-violet solar spectrum is more efficient for the growth of the plants studied than any portion of it used; the blue region of the spectrum is more efficient than the red region.

10. Light intensity is usually a limiting factor in the growth of the vegetation under a forest canopy.

11. Light quality is not a seriously limiting factor in the growth of plants under forest canopies.

12. Moderate variations in soil moisture are not likely to cause significant changes in the dry weight produced by plants provided the moisture content is not so low that it approaches the wilting coefficient, or so high that it approaches saturation.

The writer wishes to acknowledge his indebtedness to the members of the Botanical Department of Yale University for helpful criticisms of the work. He is also indebted to Dr. F. M. Schertz, associate biochemist, Bureau of Chemistry and Soils, U. S. D. A., for his kindness in furnishing a sample of crystalline chlorophyll, and for allowing the use of his method prior to its publication.

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## EXPLANATION OF PLATES

### PLATE XXVIII

- FIG. 1. The constant-condition room showing method of illumination.
- FIG. 2. A greenhouse shade showing air inlet, spreader, atmometer, and thermometer.
- FIG. 3. An outside shade showing tile air inlet, atmometer, and thermopile. The front has been removed.

### PLATE XXIX

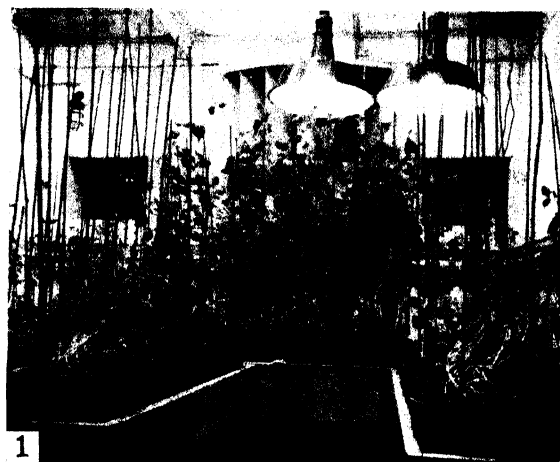
- FIG. 4. Redwood plants grown for 55 days on 12-hour days in constant-condition room. The figures above the plants show the light intensity in foot candles.
- FIG. 5. Redwood plant from the greenhouse shades, grown from March 27 to October 22, 1928. The figures on the pots show the light intensity in percentage of full sunlight.
- FIG. 6. Redwood plants grown in outside shades June 1 to Sept. 26, 1928. Figures on the pots show the light intensities in percentages of full summer sunlight.

### PLATE XXX

- FIG. 7. Sunflowers from constant-condition room grown for 48 days with 12 hours daily illumination. The figures on the pots are light intensities in foot-candles.
- FIG. 8. Sunflowers from greenhouse shades June 5 to August 13, 1928. Figures on the pots represent light intensities in percentages of full summer sunlight.
- FIG. 9. Sunflowers from outside shades June 5 to August 20, 1928. Figures on the pots represent light intensities in percentage of full summer sunlight.

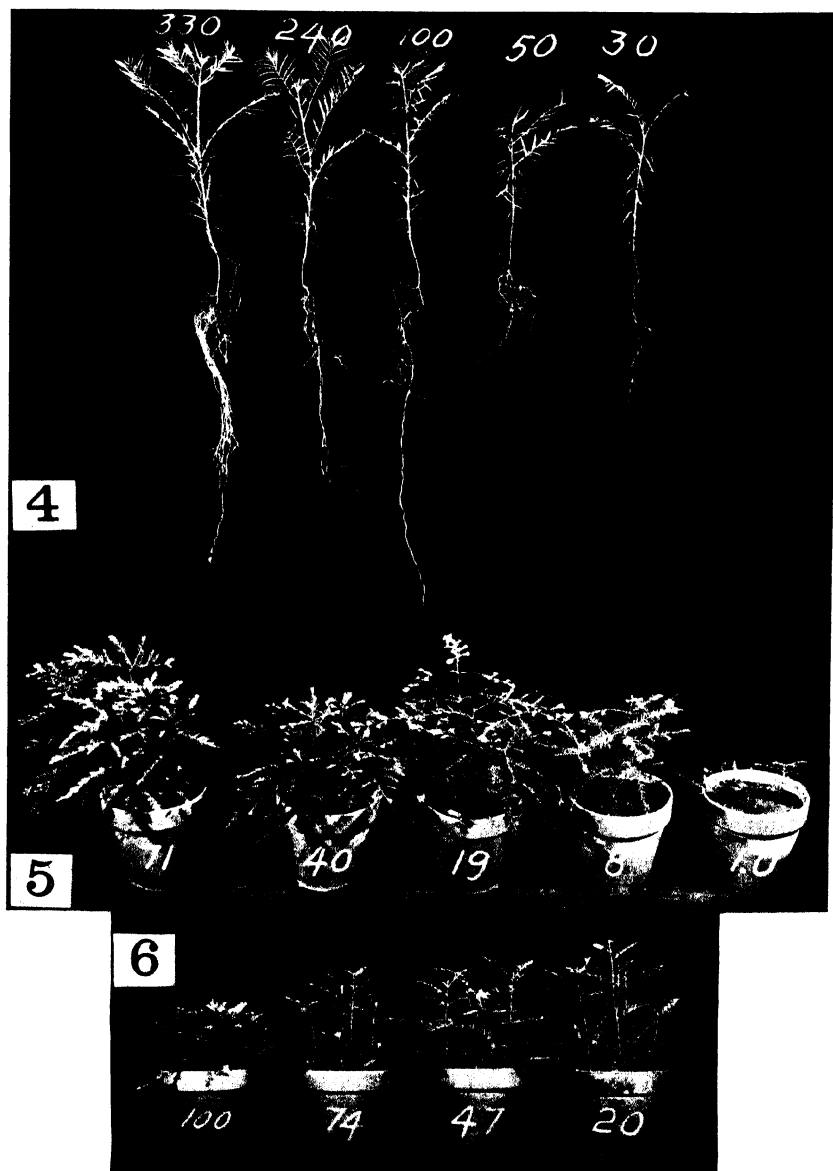
### PLATE XXXI

- FIG. 10. *Galinsoga* plants grown in the constant-condition room for 35 days with 12 hours daily illumination. Figures on the pots show the light intensities in foot-candles.
- FIG. 11. *Galinsoga* plants grown in greenhouse shades from June 5 to August 8, 1928. Figures on the pots show the light intensities in percentages of full summer sunlight.
- FIG. 12. *Galinsoga* plants grown in outside shades from June 1 to July 6, 1928. Figures on the pots show the light intensities in percentages of full summer sunlight.



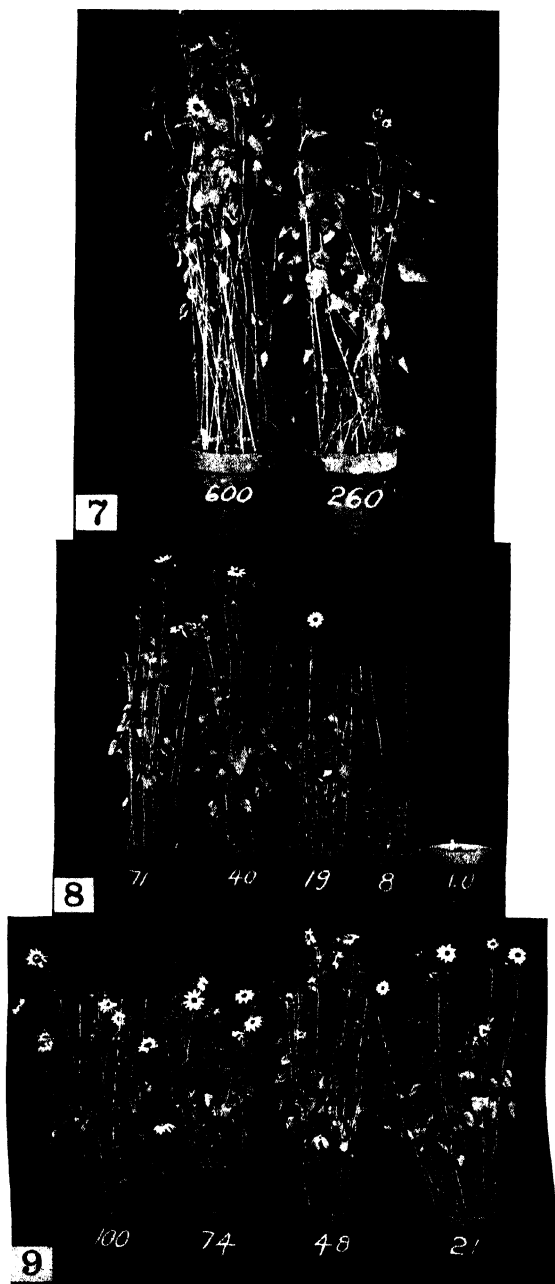
SHIRLEY: LIGHT AND GROWTH





SHIRLEY: LIGHT AND GROWTH

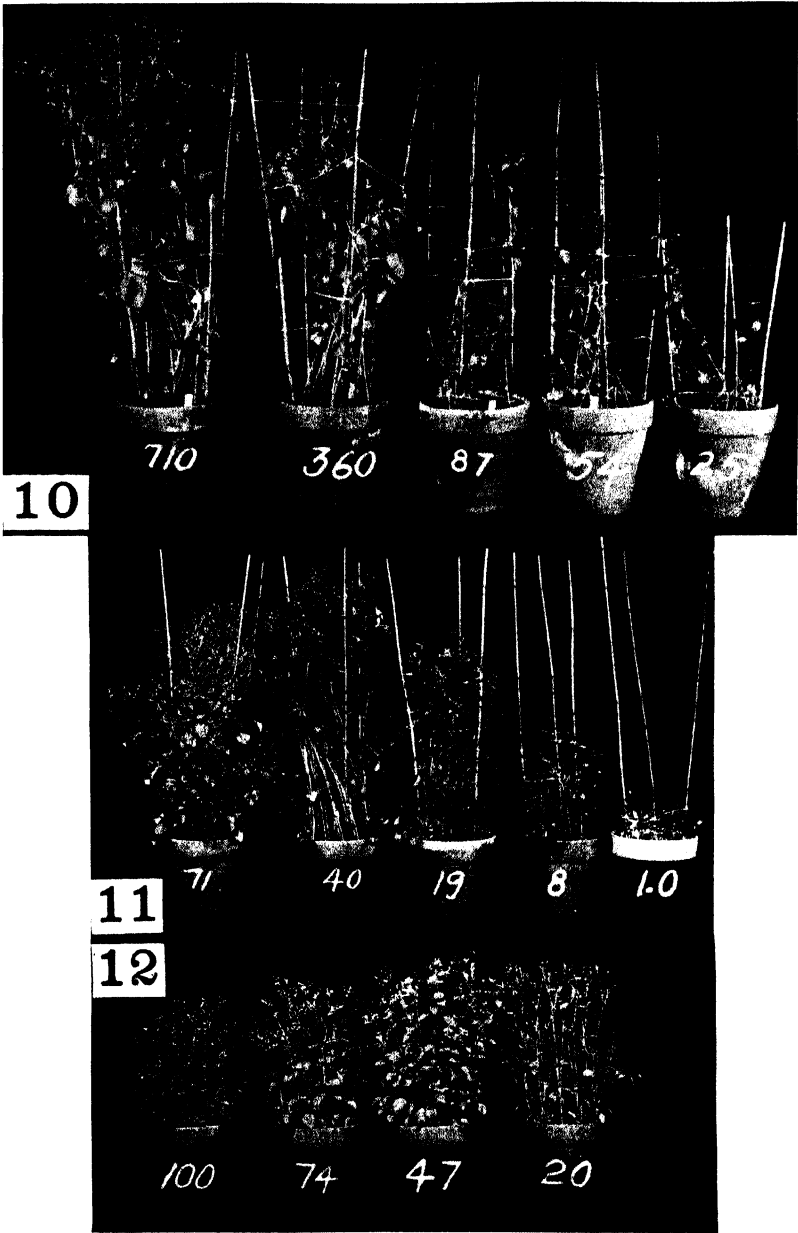




SHIRLEY: LIGHT AND GROWTH

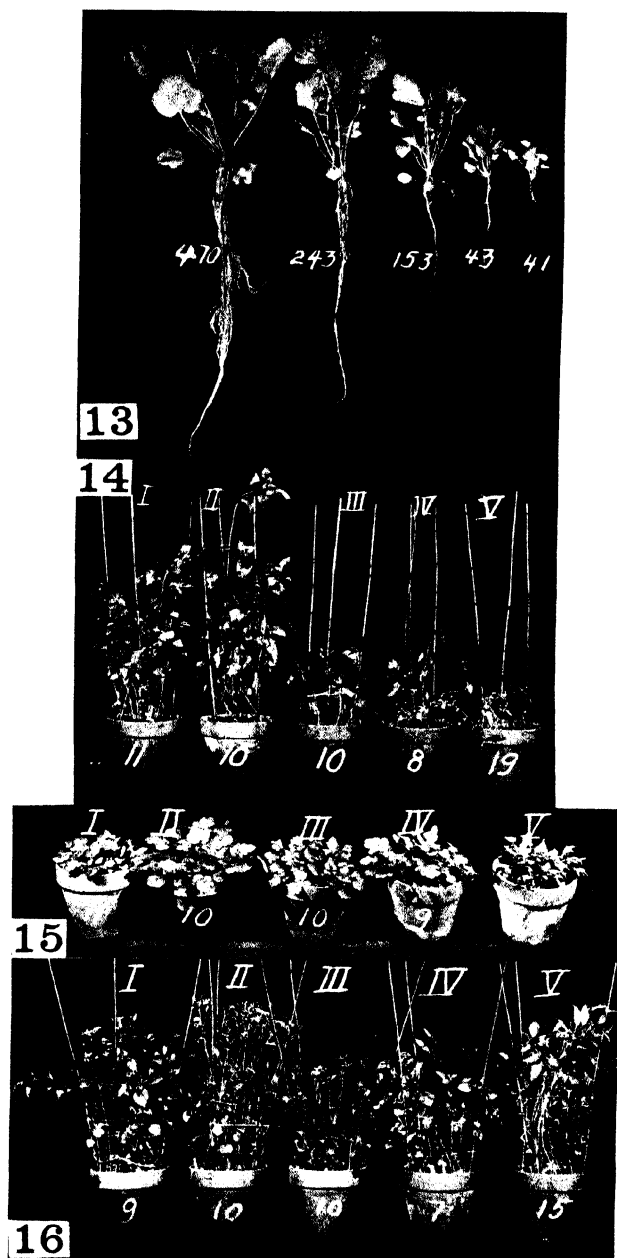






SHIRLEY: LIGHT AND GROWTH





SHIRLEY: LIGHT AND GROWTH



## PLATE XXXII

FIG. 13. *Geum* plants grown in constant-condition room for 54 days with 12 hours daily illumination. Figures on the picture show the light intensities in foot-candles.

FIG. 14. Sunflower plants grown in the spectral-house shades from June 5 to August 15, 1928. The Arabic figures on the pots show light intensities in percentages of outside sunlight. Roman numerals show the house number.

FIG. 15. *Geum* plants grown in the spectral-house shades from July 10 to October 24, 1928. Arabic figures show light intensities. Roman numerals show the house numbers.

FIG. 16. *Galinsoga* plants grown in spectral-house shades from May 30 to July 5, 1928. Arabic figures show light intensities. Roman numerals show house numbers.

## EFFECT OF CHEMICALS, TEMPERATURE, AND HUMIDITY ON THE LASTING QUALITIES OF CUT FLOWERS

A. E. HITCHCOCK AND P. W. ZIMMERMAN<sup>1</sup>

### INTRODUCTION

Numerous suggestions for methods of prolonging the life of cut flowers have appeared in journals or in books relating to floriculture. In only a few cases, however, have these suggestions been accompanied by experimental data.

Fourten and Ducomet (1) reported several chemicals as being effective in extending the life of cut flowers from 2 to 10 days beyond that of the untreated ones. Compounds were classified as favorable, indifferent, aseptic, or injurious. Some of the favorable compounds were as follows: potassium hydroxid, calcium hydroxid, potassium chlorid, potassium nitrate, potassium sulfate, ethyl alcohol, ammonium phosphate, and cane sugar. Several organic acids (acetic, oxalic, tartaric, and citric), ammonium chlorid, ammonium hydroxid, ferrous sulfate, and sodium nitrate were some of the compounds listed as unfavorable. Favorable treatments extended the life of *Primula*, *Myosotis*, *Asperula*, and *Silene* from 4 to 9 days.

Knudson (2) used many treatments in testing the effect of various compounds on the keeping qualities of cut flowers, but he was unable to substantiate the favorable results of Fourten and Ducomet. Most of the flowers which Knudson used were those having a relatively short duration of life. He also stated that zinc sulfate, a mixture of strontium and calcium chlorid, and a mixture of barium and calcium chlorids prevented the decay of African marigold and zinnia flower stems.

Laurie (3) states that each of the following chemicals prolonged the life of carnations, chrysanthemums, dahlias, and hollyhocks: a one-tenth-percent solution of boric acid, potassium permanganate, nitric acid, or potassium nitrate; a one-percent solution of cane sugar; and a solution containing one-half tablet of aspirin in two quarts of water. Asters kept twice as long in a one-tenth-percent solution of cane sugar as those which were not treated.

Perret (4) concluded that low-temperature treatment was the most effective means of preserving cut flowers. He considered relative humidity to be an important factor, the most favorable results occurring within the limits of 60 to 90 percent of saturation.

Since there is no reliable clue as to the nature of chemicals which may improve the keeping qualities of cut flowers, a selection of chemical com-

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

pounds for the purpose of making these tests must be based upon a trial-and-error method of procedure. The experiments described by the writers were planned for the purpose of testing experimentally some of the more popular claims, but in particular the effect of aspirin on the lasting qualities of cut flowers. In addition to other chemical treatments some experiments were performed to learn more about the importance of temperature and humidity. These two phases of the work are presented separately.

## CHEMICAL TREATMENTS

### Materials and Methods

*Aster*, *Coreopsis*, *Gladiolus*, *Phlox*, and *Delphinium* were placed in solutions of the compounds listed below. Not all of these compounds were used on any one variety of flower. *Dahlia*, *Cosmos*, snapdragon, *Chrysanthemum*, and *Lilium rubrum* were subjected to aspirin treatments only. The following compounds were used for the treatment of cut flowers:

- |                              |                            |
|------------------------------|----------------------------|
| 1. Acetic acid               | 27. Glycerin               |
| 2. Acetyl salicylic acid *   | 28. Iodine                 |
| 3. Alanine                   | 29. Janus green B          |
| 4. Alcohol (ethyl)           | 30. Knop's solution        |
| 5. Anilin blue               | 31. Leucine                |
| 6. Asparagine                | 32. Lithium chlorid        |
| 7. Aspirin (tablets)         | 33. Magnesium acetate      |
| 8. Atropine                  | 34. Nicotine               |
| 9. Barium acetate            | 35. Nu-green               |
| 10. Barium chlorid           | 36. Potassium ferricyanid  |
| 11. Barium hydroxid          | 37. Potassium permanganate |
| 12. Belladonnine             | 38. Quinine                |
| 13. Calcium acetate          | 39. Safranin               |
| 14. Calcium carbonate        | 40. Sodium acetate         |
| 15. Calcium hydroxid         | 41. Sodium bicarbonate     |
| 16. Calcium oxalate          | 42. Sodium carbonate       |
| 17. Calcium salicylate       | 43. Sodium chlorid         |
| 18. Camphor †                | 44. Stout's solution §     |
| 19. Cupric acetate           | 45. Strontium acetate      |
| 20. Cupric chlorid           | 46. Strontium chlorid      |
| 21. Cupric nitrate           | 47. Strontium nitrate      |
| 22. Copper sulfate           | 48. Sulfanilic acid        |
| 23. Cobalt acetate           | 49. Sulfurous acid         |
| 24. Florocein                | 50. Uspulun                |
| 25. Flower food (plant food) | 51. Xylol                  |
| 26. Gallic acid              |                            |

\* Acetyl salicylic acid is the principal constituent of aspirin tablets.

† One-fourth teaspoon to 1 quart of water, one-half and one-fourth strength.

§ One tablespoonful each of table salt, sodium bicarbonate, and ammonia water in 1 quart of water, and 1 tablespoonful of this mixture in 1 pint of water.



Preliminary tests were first run to determine the limits of decidedly injurious concentrations. Dilutions of one-fifth, one-half, or one-tenth were made from 1- to 10-percent solutions until concentrations of from 0.001 to 0.000001 percent were reached. Repetition of treatments was then confined to concentrations which were slightly injurious and to more dilute ones. Solutions were usually placed in graduates, both to facilitate dilution procedure and to determine the amount of water used by the flowers. Tap water was used in most of the experiments since no difference could be detected between the response of flowers in tap water and that in distilled water. The use of solvents other than water was sometimes necessary, but in such cases a solution of the pure solvent was used as a check in addition to a water check. Flowers with a similar external appearance were regarded as being comparable. In some of the experiments flowers were used which had opened from bud within 16 or 24 hours' time.

### Experimental Results

None of the compounds was noticeably effective in prolonging the life of cut flowers used in these experiments. Many of the solutions in concentrations lower than 0.25 percent were either beneficial or non-toxic to the flowers. *Coreopsis* in 0.5- to 2.5-percent ethyl alcohol, for example, was usually better than the check. Most of the chemicals, however, were toxic in concentrations greater than 0.25 percent. In concentrations greater than 0.01 percent the following compounds produced injury consistently: acetyl salicylic acid (both aspirin tablets and the chemically pure salt), cupric acetate, cupric chlorid, cupric nitrate, copper sulfate, cobalt acetate, calcium salicylate, lithium chlorid, nicotine, nu-green, potassium ferricyanid, sulfanilic acid, and uspulun. Xylol was toxic when added at the rate of 2.5 cc. per 250 cc. of water. Copper salts were sometimes injurious at a concentration of .001 percent, the same variety of flower responding differently at one time than at another.

Injury was not always in the form of wilting or browning of petals and stems. Changes in petal color resulted from treatment with acetates of copper, barium, strontium, magnesium, and sodium; chlorids of lithium, barium, strontium, and sodium; sodium carbonate, sodium bicarbonate, barium hydroxid, sulfurous acid, and anilin blue in concentrations of 0.5 to 0.125 percent. The two carbonates and lithium chlorid produced the most marked fading of pink portions of *Gladiolus* petals. Lithium chlorid was the most effective in causing the lower dark red portions of *Gladiolus* petals to turn deep purple. Flowers undergoing pronounced color changes did not last as long as the checks, but those showing slight color changes were comparable with the untreated lots except for depth of color. Even though the four copper salts in concentrations greater than .05 percent usually injured the stems of *Gladiolus*, the petals would often remain in good condition as long as those of the untreated flowers.

The effect of a particular compound was not always the same for different varieties of flowers. *Phlox*, for example, was injured by a 0.003-percent solution of gallic acid, whereas it required a 0.1-percent solution to injure *Delphinium*. Although a 1-percent solution of copper sulfate injured aster stems badly, *Phlox* stems were unaffected by the same treatment. Flowers of *Lilium rubrum* were injured by a 0.003-percent solution of sodium chlorid, while a concentration greater than 0.05 percent was required to injure *Coreopsis*.

Stems of *Aster* and *Phlox* were preserved in excellent condition when placed in a 0.5- to 0.05-percent solution of potassium permanganate. Higher concentrations of permanganate were equally effective when the flowers were transferred to water after a one-hour treatment.

### Discussion

Although many treatments appeared to be beneficial, the variation in response among lots receiving similar chemical treatment at different times was as great as that between treated and untreated lots. A similar variation occurred among untreated lots of the same variety of flowers which were taken at different times. In view of this variation the beneficial results for individual treatments can not be considered as significant, with the possible exception of the ethyl-alcohol treatment. Since *Phlox* and *Coreopsis* usually lasted from 1 to 2 days longer when placed in ethyl alcohol than similar untreated lots, this treatment must be considered as slightly favorable.

The use of potassium permanganate is certainly one means for preventing the rapid decay of *Phlox* and *Aster* flower stems, but in these experiments the floral parts were not benefited in proportion. The comparatively short time which asters lasted may be accounted for in part by the fact that practically all plants from which these flowers were taken had aster yellows.

In the early experiments no attempt was made to control temperature or humidity, but in other later experiments these two factors were found to be of considerable importance. Individual variation among a given lot of flowers receiving the same treatment was such that some would remain in perfect condition for from one to several days longer than others, even though all flowers had opened from bud within the same period (16 or 24 hours) and were selected to be comparable in external appearance.

The possibility of beneficial chemical treatment of cut flowers is by no means precluded by these or similar experiments, but certainly the results were not sufficiently striking to warrant the use of such compounds as aspirin, sodium carbonate, sodium bicarbonate, and sodium chlorid. As Knudson pointed out, a favorable treatment would be expected to slow up the normal maturing processes that make for seed production and the consequent loss of floral parts. Since it is known that low temperature (3° to 10° C.) will retard the maturing processes in flowers, many attempts

have been made to find a chemical treatment which would produce similar results at room temperature without at the same time causing injury.

The fact that anesthetics will retard certain metabolic processes in man has been partly responsible for attempts to find a chemical treatment which will "put flowers to sleep" very much the same as ether will anesthetize human beings. Since low temperature may be regarded as a favorable treatment, and since it retards transpiration, it might be expected that a favorable chemical treatment would likewise reduce water loss from floral parts. In these experiments transpiration was reduced by many of the chemical treatments, but in all cases the flowers which remained in the best condition were those that lost the greatest amount of water daily. Thus the relative amount of water lost was an index to the condition of the flowers.

#### EFFECT OF TEMPERATURE AND HUMIDITY

##### Materials and Method

Cut flowers of carnation, rose, *Cosmos*, and *Dahlia* were subjected to different temperatures and humidities. Flowers placed under bell-jars received air which was first passed through a series of three 1-liter flasks containing a known concentration of reagent quality sulfuric acid. By varying the concentration of sulfuric acid it was thus possible to provide a series of different vapor pressures. Vapor-pressure calculations were taken from Wilson's chart (6) and the resulting relative humidities were measured in the bell-jars with Shippy's modified wet and dry bulb apparatus (5). "Petticoat bubblers" were used in each flask in order to insure the attainment of equilibrium relations between the air drawn through and the sulfuric acid solution.

Laboratory temperature was decidedly variable (22° to 34° C.). Refrigeration rooms, however, were kept constant at 20°, 15°, 10°, and 5° C. Humidity experiments were run at room temperature, 15°, and 10° C. Four varieties of carnations were used (Morning-low, Supreme, Ward, and a yellow variety). Briarcliff Rose, Jersey's Beauty *Dahlia*, and both the early and late flowering *Cosmos* constituted the other varieties of flowers used. Carnations and roses were purchased from a local florist. All other varieties of flowers were grown on the Institute grounds.

Final data were taken for each treatment when the majority of the flowers ceased to remain in good condition. The total time lasted does not include the day on which final data were taken.

##### Results

The effect of constant temperature on the keeping qualities of cut flowers is shown in table 1. Data concerning alternating temperatures are given in table 2. *Coreopsis*, for example, lasted four times as long at 5° C. as at room temperature. *Cosmos* was not so greatly benefited by low tempera-

TABLE 1. *Effect of Temperature on Coreopsis and Cosmos*

Temperature in ° C.	Number of Days Flowers Lasted				
	<i>Coreopsis</i>			<i>Cosmos</i>	
	Lot I	Lot II	Lot III	Lot I	Lot II
Room (22–34° C.).....	4	3	2	3	3
20.....	5	3	3	2	3
15.....	7	7	5	2	4
10.....	12	8	9	3	5
5.....	18	13	14	3	5

TABLE 2. *Effect of Alternating Temperature on Coreopsis*

Temperature Conditions in ° C.	Number of Days Flowers Lasted	
	Lot I	Lot II
Room during day, 5° at night.....	6	6
“ “ “ 10° “.....	6	6
“ “ “ 15° “.....	4	4
“ “ “ 20° “.....	4	2
5° “ “ 10° “.....	12	—
10° “ “ 5° “.....	13	—
5° “ “ , room “.....	3	4
24 hours at room, 24 hours at 5°.....	—	8
5° 2 days, rest of time at room.....	—	5
5° 3 “ “ “ “ “ “.....	—	6

ture. When flowers of *Coreopsis* were kept at room temperature during the day and at 15° or 10° C. during the night, a gain of only 2 days resulted. Alternating between 5° and 10° C. day and night, respectively, was the same for *Coreopsis* as a constant 10° C. temperature. A gain of 2 days over the day and night alternation resulted when the flowers were kept 24 hours at room temperature and 24 hours at 5° C. *Coreopsis* stored at 5° C. for 2 and 3 days lasted an additional 3 days when removed to room temperature. Similar lots placed at room temperature from the beginning also lasted 3 days. After 7 days' storage at 5° C. *Coreopsis* and *Gladiolus* wilted in a short time when transferred to room temperature, *Coreopsis* lasting one day and *Gladiolus* lasting only 3 hours. Both lots were in perfect condition at the time of transfer, at least so far as external appearance was concerned.

Data showing the effect of relative humidity at different temperatures are given for carnation in table 3, and for rose in table 4. Carnation showed a consistently favorable response not only to a relatively high humidity, but to a humidity of 98 percent as compared with one of 80 percent. Low temperature treatment was also decidedly beneficial to the carnation, especially at a high humidity. It must be noted, however, that carnation kept as well at the lowest humidity (15 percent) in the 10° C. room as in a saturated atmosphere at room temperature.

TABLE 3. *Effect of Temperature and Humidity on Carnation*

Relative Humidity	Number of Days Flowers Lasted									
	Room Temp. (22-34° C.)				15° C.		10° C.			
	Lot No.				Lot No.		Lot No.			
	I	II	III	IV	I	II	I	II	III	IV
Air.....	3	1	1	2	5	3	5	3	2	3
15.....	2	1	1	2	4	2	6	3	2	2
55.....	5	2	1	4	4	2	8	4	2	3
80.....	5	2	1	4	6	5	11	5	8	11
98 +.....	5	4	2	5	12	9	12	9	12	12

TABLE 4. *Effect of Temperature and Humidity on Rose (var. Briarcliff)*

Relative Humidity	Number of Days Flowers Lasted					
	Room Temp. (22-34° C.)		15° C.		10° C.	
	Lot I	Lot II	Lot I	Lot II	Lot I	Lot II
Air.....	3	4	5	3	9	10
15.....	3	4	5	2	8	10
55.....	3	5	7	2	12	10
80.....	3	5	7	5	8	9
98 +.....	3	5	6	9	9	10

Roses lasted 2 to 3 times as long at 10° C. as at room temperature but they were not particularly benefited by a high humidity. *Cosmos* and *Dahlia* were variable, some lots lasting best at high, and other lots at low humidities.

### Discussion

Low temperature treatment was beneficial in practically all cases, but especially so for *Coreopsis* and carnation. Alternating high and low temperatures do not appear to be so effective on a day and night alternation as on a 24-hour alternation. Flowers stored at 5° C. for 7 days or longer may be expected to wilt much sooner when removed to temperatures above 22° C. than a fresh lot of flowers.

The behavior of *Dahlia* was somewhat puzzling. Jersey's Beauty dahlias placed at 5° C. remained in perfect condition for 14 days, yet similar lots placed at 10° C. lasted only 4 days. It is to be noted, however, that according to the results given in tables 3 and 4 there was a considerable difference in response of flowers placed at 10° C., 15° C., and room temperature. In the case of *Coreopsis* (table 1) 5° C. was much more favorable than 10° C.

In the case of carnation there was a close correlation between the degree

of relative humidity and the lasting quality of the flowers. This relation, however, was noticeably modified by temperature. While it is to be expected that all kinds of flowers will not respond similarly to a given set of temperature and humidity conditions, humidity is nevertheless important and may be a limiting factor even at 10° C.

### SUMMARY

#### *Chemical Treatment*

1. None of the 50 chemical compounds used was noticeably effective in prolonging the life of cut flowers.
2. Within a restricted range of concentrations certain compounds were toxic and others were not. Injury was manifested in *Gladiolus* as wilting and as a color change in the petals.
3. Potassium permanganate was consistently effective in preventing the decay of the flower stems of *Phlox* and *Aster*, although for the floral parts no marked gain in time of lasting resulted from this treatment.
4. Variation among individual flowers was such that in check lots as well as in treated ones some of the flowers would be in good condition after a few days while some would be badly wilted or dead.

#### *Effect of Temperature and Humidity*

1. Low temperature (5° to 10° C.) was especially favorable for keeping cut flowers of coreopsis, carnation, and rose. *Dahlia* and *Cosmos* were not so greatly benefited at 10° C.
2. Carnations kept 2 to 3 times as long in an atmosphere which was nearly saturated as they did in a humidity below 80 percent of saturation. The other varieties of flowers were not so greatly benefited by high humidity treatments.
3. Carnations lasted longer at low humidities if the temperature was also low, but humidity was a limiting factor even at 10° C.

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## VEGETATIVE PROPAGATION OF HOLLY

P. W. ZIMMERMAN AND A. E. HITCHCOCK

Propagation of hollies has been considered an important subject in recent years because of extensive ravages made upon these plants at Christmas time and also because of their growing popularity as ornamentals for home planting. Nurserymen have not been able to supply the demand for plants since seeds have germinated very poorly and cuttings have not responded well. An occasional cutting has been rooted from time to time by some growers but their success has been far too infrequent to be of any commercial importance. Hollies are dioecious, and due to the fact that berried types can not be selected from seedlings until the plants are several years old vegetative reproduction is of particular value. There is also much variation in leaves, berries, and size among seedlings, and if a good method for vegetative propagation can be found, new plants which would grow true to type could be made from selected trees.

Cutting material was taken from one deciduous and five evergreen species of holly, though most of the experimental work was done with one evergreen variety, *Ilex opaca*. The results reported in this paper are concerned with the following topics on vegetative propagation of holly: the percentage of rooting from cuttings; the variation in response of material from different trees; seasonal effects; the age of the tissue as affecting rooting; variation according to rooting media used; leaves as necessary organs on cuttings of evergreen varieties; light requirements; temperature and time required for rooting; position of roots; chemical changes while the cuttings are in the rooting medium; and the growth of holly plants which have been produced from cuttings.

### MATERIAL AND METHODS

*Source of Holly Material.*—For the most part materials used in the experiments came from Maryland, New Jersey, or Massachusetts. Smaller collections have come from the New York Botanical Garden, Pennsylvania, Oregon, and California.

*Selection of Cutting Material.*—Material with little or no leaf-spot disease was made into three types of cuttings as follows: (1) current year growth cut to eliminate the old wood; (2) current year growth plus enough two-

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y., and the Maryland Agricultural Experiment Station, College Park, Maryland; published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.



year-old wood to furnish cuttings four to five inches in length, and (3) culls which were generally discarded but were used in a few cases to determine the rooting capacity of old wood. The basal portions of culls were generally two to four years old.

The uppermost three or four leaves were left on the cuttings. It was impossible to make the cuttings so as to leave exactly the same amount of leaf area on each specimen. An attempt was made to approach uniformity by removing whole leaves or parts of leaves.

*Time of Year.*—The experiments reported in this paper were conducted with material collected between August and January inclusive. Rooting was equally good any time between August and December 31. Thereafter there was some variation according to weather conditions which may cause winter injury.

*Media Used.*—Water-washed sand, bank sand, peat moss, slag, and mixtures of peat moss and sand, constituted the media used. The water-washed sand was neutral in reaction; the bank sand had a pH value of 5.5 to 6, the slag was alkaline with a pH value of 8 to 9, and the peat moss showed a pH of 3.6 to 4.0. When peat moss was used with sand, 50 percent of each by volume, the mixture generally gave a pH value of 4.5. The medium was approximately six inches deep in the bench.

*Method of Planting Cuttings.*—The cuttings were planted so that the leaves rested on the medium. It was generally necessary to slant the cuttings to make the leaves lie flat and also to keep the base of the stem away from the boards holding the medium. Usually the basal portions of the lowermost leaves dipped into the medium. With this method it was necessary to remove the cutting from the medium to observe root development.

*Time Required.*—Some cuttings root in three weeks' time, but to obtain a high percentage of rooting it is necessary to leave the cuttings in the medium for three to four months. Plants that are to be potted should have a large root system which will not break easily. To obtain such results requires approximately 14 weeks.

*Temperature.*—Evergreen hollies were grown in greenhouses with a temperature range of 60° to 75° F. On clear days the temperature often went above 75° F. *Ilex verticillata* was grown in constant-temperature incubators maintained at 80.6°, 75.2°, 68°, and 59° F. While in the incubators the cuttings were surrounded with moist sphagnum moss either in long glass tubes or in clay pots. When in glass tubes the basal portion of the cutting was placed so that it could be observed without being removed from the medium. With this method roots could be seen within a short time after they emerged from the bark.

*Handling of Holly Plants.*—When it was desirable to grow holly plants which had been produced from cuttings, they were potted and placed in Wardian cases (board cases with glass covers) until new roots had started

to grow. The cases were used for the purpose of maintaining a high relative humidity about the newly potted plants. After three weeks in the cases holly plants were set on the bench and handled like the average potted plants.

Holly does not require a special type of soil. It does especially well, however, in leaf mould or a mixture of peat moss and soil.

Potted plants may be planted out of doors any time during spring or early summer. If new shoots arise after July 15 they are apt to be injured by cold weather during the winter.

## RESULTS

### Variation in Rooting of *Ilex opaca* Cuttings According to the Source of Material

Table I shows the results of experiments with cuttings from four different trees growing in Massachusetts. The cuttings were all in the same green-

TABLE I. *Variation in Rooting Response of Holly (I. opaca) Cuttings Taken from Four Different Trees in Massachusetts. The Cuttings Were Placed in a Medium of 50 Percent Peat Moss and 50 Percent Sand on Oct. 28, 1927 and Left Until Feb. 6, 1928*

Tree Designation	Type of Cutting	Total No. Cuttings	Percentage Rooted	Percentage Dead
Tree no. 0. . . . .	Current growth	161	39.5	7.5
" " 1. . . . .	"	46	59	15
" " 2. . . . .	"	67	80	2
" " 3. . . . .	"	88	81.5	9
Tree no. 0. . . . .	Current growth plus a portion of old wood	147	47	6.6
" " 1. . . . .	"	117	45	33
" " 2. . . . .	"	316	73.2	8.5
" " 3. . . . .	"	60	78	14
Tree no. 0. . . . .	All types, including culls	1,001	47.7	5.3
" " 1. . . . .	"	244	51.7	22
" " 2. . . . .	"	844	73.3	7.7
" " 3. . . . .	"	685	65	21.9

house and grown in a mixture of 50 percent peat moss and 50 percent sand. The experiments were started on October 28, 1927 and ended February 6, 1928.

Table 2 shows the results with material from nine different trees growing in the woods near New Lisbon, N. J. Cuttings from trees labeled 1, 2, 3, 4, and 5 were taken on October 4. Those labeled *a*, *b*, *c*, and *d* were taken December 18. Greater variation occurs with cuttings taken in January and February, due probably to frost injury.

### Winter Injury to *Ilex opaca*

Two lots of holly were brought from Massachusetts in 1927 and 1928. The first lot was collected and made into cuttings on October 28, 1927.

TABLE 2. *Variation in Rooting of Holly (I. opaca) Cuttings Taken from Different Trees Growing in the Woods Near New Lisbon, N. J.*

Tree Designation	Date Taken	Date Exp. Was Concluded	Rooting Medium	Type of Cutting	Total No. Cuttings	Percentage Rooted *	Percentage Dead *
Tree no. 1..	10/ 4/28	3/10/29	Slightly acid sand	All types included	150	68.7	24.9
" " 2..	"	"	"	"	320	51.7	21.2
" " 3..	"	"	"	"	155	60	24.5
" " 4..	"	"	"	"	91	74.7	14.3
" " 5..	"	"	"	"	136	78	19.1
Tree no. a..	12/18/28	2/20/29	50-50 peat and sand	Current growth	61	72	3.3
" " b..	"	"	"	"	59	64	0
" " c..	"	"	"	"	60	66	1.6
" " d..	"	"	"	"	61	79	0
Tree no. e..	12/17/28	4/ 3/29	Slightly acid sand	"	60	61.6	3.3
" " f..	"	"	"	"	60	53.3	3.3
" " g..	"	"	"	"	60	36.6	31.6
" " h..	"	"	"	"	60	45.0	28.3

\* The percentage figures show the average of all lots for each tree.

The results are shown in table 1. The second lot was collected about January 15, 1928. The leaves showed signs of frost injury and withered quickly after being placed in the rooting medium. There was practically a 100-percent loss in the lot.

Two collections of cuttings were made from trees near New Lisbon, N. J., one in September 1927 and the other in January 1928. The first lot was unusually satisfactory, rooting as high as 90 percent. The second lot showed some frost injury and leaves were soon lost after the cuttings were placed in the medium. As with the Massachusetts cuttings they were nearly a total loss.

Several lots from New Lisbon, N. J. in October 1928 and December 1928 were about alike in response. Data showing the number of cuttings and the percentage of rooting from these lots are given in table 2. As during the previous year, however, cuttings brought from the same locality near the end of January 1929 soon showed yellowing of the leaves and were discarded.

The failure to root seems to be due to winter injury rather than mere seasonal changes in the composition of the stems or leaves, since cuttings taken after January 5, 1927, in Maryland gave consistently good results. Cuttings from Maryland in 1928 did very poorly after January 15. The leaves showed about the same type of winter injury as was characteristic for the Massachusetts and New Jersey holly.

### Age of Cutting Material Used for *Ilex opaca*

Succulent material of *Ilex opaca* taken in May and June did not root. Current-year stems root equally well from August to January or until winter injury occurs. As shown by data in table 1 two-year-old wood is nearly as effective as current growth. Culls with stems three or more years old produce a high percentage of rooting. The table shows some advantage for current-year stems when all older wood has been removed, the percentages being 80 for current-year stems and 73 where a portion of one-year-old stems remained attached. Results for the two types were not always consistent, but where large numbers were used the percentage of rooting was higher for the current-year stems.

### Effect of the Medium

Five different media were used as follows:

1. Imported German peat moss (pH 3.6 to 4.0)
2. Bank sand (pH 5.5 to 6)
3. Water-washed sand from Long Island Sound (pH 7)
4. Equal portions of sand and peat moss mixed (pH 4 to 5)
5. Slag from steel mills (pH 8 to 9)

TABLE 3. Media Tests with Cuttings of Holly (*Ilex opaca*)

Tree Designation	Dates	Medium *	No. of Cuttings	Percentage Rooted	Percentage Dead
Tree no. 1 N.J. ....	10/4 to 12/13/28	Mixture	18	88.8	0
" " " " ....	"	Bank sand	17	70	0
Tree no. 2 N.J. ....	"	Mixture	18	66.6	0
" " " " ....	"	Bank sand	17	53	0
" " " " ....	"	Slag	20	0 †	Not counted
Trees no. a & d N.J.	12/18/28 to 2/2/29	Mixture	10	33.3	10
" " " "	"	Bank sand	10	38	30
Tree no. 1 Md. ....	10/15/25 to 3/15/26	Peat moss	20	90 †	10
" " " " ....	"	Mixture	20	75	0
" " " " ....	"	Bank sand	20	50	0
Maryland. ....	1/5 to 4/15/27	Peat moss	40	50	—
" " " " ....	"	Mixture	40	71	—
" " " " ....	"	Bank sand	40	30	—

\* The word "mixture" refers to a mixture of 50 percent peat moss and 50 percent sand.

† The cuttings in slag remained in the medium until March 15, 1929 at which time 20 percent had rooted and 70 percent were dead.

† The peat moss was 3 inches deep over sand. The ends of the cuttings were in or near the sand.

All cuttings used for media tests were taken from the same tree so as to reduce possible variation. Rooting occurred in all the different media but the percentage varied with the media. Table 3 shows the results with pure peat moss, mixtures of peat moss and sand, bank sand, and slag which is a waste product from the steel mills. The percentage of rooting varied in the different lots. In one case the lots in sand gave a slightly higher percentage of rooting than those in a mixture of peat moss and sand. In another case pure peat moss was better than the mixture but in three cases out of five the mixture was the best medium. Possible reasons for such variations are mentioned in the discussion.

### The Value of Leaves on Cuttings

In 1927 all leaves were removed from five lots of 20 cuttings each, and they were then placed in a mixture of peat moss and sand. They did not root even though left for a longer time than is necessary for the average leafy cutting to respond.

*Ilex verticillata*, a deciduous holly, roots readily from hard-wood leafless cuttings. In 1925 ten cuttings of *I. verticillata* formed roots about one inch long around the base of the stems. The lower ends of the cuttings were removed so as to eliminate all the roots, and a second set of roots, as vigorous as the first, appeared in a short time. Cuttings of evergreen varieties failed to root after the leaves were removed. The minimum number of leaves necessary for a cutting to root has not been determined, but probably as many as can be kept in good condition will always work to the advantage of the cutting.

### Light Requirements

*Ilex verticillata* cuttings can be rooted in dark chambers, but evergreen species need light while in the rooting medium. The exact amount of light required has not been determined. Six hours of extra light at night from 1000-watt nitrogen bulbs has been beneficial to root growth and in some cases to the percentage of rooting. Plate LV, figure 1, shows two lots of *Ilex crenata* which received different amounts of light. The lower row was given normal sunlight from November 16, 1928 to January 9, 1929. The top row during the same period was given six hours of artificial light in addition to normal sunlight. There was an increase in both the percentage of rooting and in the size of the roots. All of the cuttings receiving extra light rooted, while only 45 percent of those receiving normal light formed roots. Also new shoots were appearing under extra light treatment while cuttings receiving normal sunlight remained dormant.

Plate LV, figure 2 shows two rows of *Ilex opaca* which received different amounts of light from October 4 to December 13, 1929. The top row was given 6 hours of artificial illumination in addition to full normal sunlight;

the lower row was given only normal light. Not all lots of *I. opaca* in extra light have shown an increase in percentage of rooting, but the size of roots produced was always larger than in normal light.

In the case of *Ilex glabra* the extra light was beneficial for both root growth and percentage of rooting. In two lots of 24 cuttings each, 16 rooted in extra light while only 11 rooted in normal light. The experiment was repeated twice with similar results. The difference in the size of the roots was more striking than the difference in percentage of rooting.

### Temperature and Time Requirements

Constant-temperature incubators could not be used for evergreen varieties since light is necessary for root growth. Deciduous varieties, however, root while in the darkness, and table 4 shows the effect on *Ilex*

TABLE 4. *The Effect of Constant Temperatures on Time Required for Rooting of Ilex verticillata. Experiment was Started on January 15, 1925. Each Lot Included 10 Cuttings. The Data Were Taken after All Cuttings in Each Lot Showed Roots*

Temperature	Time
59° F.....	42 days
68° F.....	28 "
72.5° F.....	21 "
80.6° F.....	18 "

*verticillata* of three constant temperatures maintained in incubators. The cutting material was uniform and the response was nearly uniform, though a few days elapsed between the time the first roots could be seen until the last of the lot had rooted. No rooting occurred at 50° F. At temperatures higher than 95° F. the cuttings rotted before they had enough time to form roots. The optimum for rooting is probably somewhere between 75.2° and 80.6° F. For practical purposes, however, it might be best to grow the cuttings at approximately 68° F., since less loss from decay is likely.

Evergreen varieties have been grown at variable temperatures in the greenhouses and no accurate data on temperature effects could be collected. An effort was made to keep the night temperature at 60° F., and the day temperature not to exceed 75° F., though when the sun was bright the temperature often mounted to 85° F. It is probable that the evergreen varieties, like the deciduous ones, can produce roots at any temperature between 58° and 85° F., but that the time required will vary with the temperature.

In several collections of *Ilex opaca* taken between August and December some rooting occurred in 21 days. Variation in time of response is well illustrated by Plate LVI, figure 3, showing a lot of 16 holly cuttings from New Jersey. They were placed in the medium on December 17, 1928, removed and photographed on February 20, 1929, a total of 65 days. This illustrates as favorable a response as could be hoped for in *Ilex opaca*, but

it shows variation in time of rooting although the cuttings were given the same treatment as far as known. The seven cuttings on the left probably had rooted in three or four weeks, the next four in five or six weeks, and the next four in six or eight weeks. One cutting on the right, though in good condition, did not root in 65 days. This illustrates approximately what may be expected in any collection of holly cuttings if taken from a favorable tree any time between August and January 1. If plants are to be grown from cuttings the root system should be approximately as large when potted as those shown on the seven cuttings to the left. To obtain such rooting on the majority of the cuttings it was necessary to leave them in the medium three to four months.

*Ilex cornuta* responded in about the same time required for *I. opaca*. On December 30, 1927, 99 cuttings were placed in bank sand and on April 14, 1928, 97 of the cuttings had rooted, one was in good condition but not rooted, and one was dead.

In one lot of 20 cuttings of *Ilex crenata*, 9 specimens rooted in 40 days (October 4 to November 13). Approximately 50 percent of *Ilex glabra* cuttings started on January 16 rooted in 60 days.

### Position of Roots

The majority of *I. opaca* cuttings produced roots just above the cut surface. Occasionally a few roots originated along the stem or from the callus. This occurred particularly in young stems taken from plants in New Jersey. *Ilex cornuta*, *I. glabra*, and *I. crenata* send out their roots very much like *I. opaca*, but *I. verticillata* has been observed to send out roots only from the bark just above the cut surface.

### Carbohydrate Storage

*Ilex opaca* cuttings were gathered in New Jersey on October 5, 1928 and the stems were tested microchemically on October 6. Only traces of starch could be found in any of the stems tested, but the reducing substances were fairly plentiful.

The cuttings were then placed in the rooting medium under two different light conditions: (1) normal sunlight, and (2) normal sunlight plus six hours of light from 1000-watt nitrogen bulbs. On December 15 approximately half of the cuttings had rooted. At this time the tests showed an abundance of starch in rooted cuttings and a fair amount in non-rooted cuttings. A few of the non-rooted cuttings had more starch than some of the rooted cuttings. Also the stems in extra light had slightly more starch than those in normal light.

Sugars which were fairly plentiful at the beginning were present only in traces in both sets at the end of the experiment. It appears that the starch increased while reducing substances decreased. Collections of holly

from the same locality taken December 18 were fairly well supplied with starch and reducing substances. After remaining in the medium until April 12 they showed an increase in starch but a great decrease in reducing substances. At this time one non-rooted cutting which was tested had a fair amount of starch and a good supply of sugar. Cuttings taken from trees in New Jersey on February 5 had an abundance of starch. From these data it appears that the stems of *I. opaca* accumulate starch throughout the months of October to February.

*Ilex crenata* cuttings were collected and tested on November 16 and then placed in the rooting medium in the greenhouse and in three dark places at 41°, 50°, and 59° F. They were tested bi-weekly thereafter. At the beginning the pith of the stems was full of starch. The lot in the greenhouse kept an abundance of starch in the pith and after four weeks the cortex also was full of starch. About 50 percent of the cuttings had rooted by January 9 but no difference could be detected in the starch content of rooted and non-rooted specimens.

The starch disappeared regularly from the cuttings stored in darkness at 41°, 50°, and 59° F. After 6 weeks none could be found in the 50° and 59° F. lots. At the same time a fair amount of starch was present in the 41° F. lot. No rooting occurred in the lots stored in darkness.

### Growth of Plants from Cuttings

Approximately 2000 holly plants (*I. opaca*) resulting from cuttings taken in October, 1927, failed to grow normal shoots after being held continuously through the winter at approximately 70° F. Though the cuttings formed good roots the buds did not grow. During the summer of 1928 a few new shoots arose though in most cases they were from lateral buds instead of terminals. Also buds on two-year-old wood frequently developed into new shoots. The terminal buds did not grow until after they had passed the winter of 1928–1929 in a cold house. On April 9, 1929, practically every plant was producing normal shoots from terminal and lateral buds characteristic for holly. Even flower buds came through the long dormant period without apparent injury (Pl. LVII, fig. 5).

Cuttings taken from trees after December 15, 1927, developed roots by March, 1928, and new shoots during April and May. Of 180 plants resulting from cuttings taken in October, 1928, only 17 new shoots had appeared on April 9, 1929. Of the 200 plants from cuttings taken from the same locality December 17, 1928, all were developing normally on April 9, 1929. Apparently the cool weather from October to December 17 was sufficient for "after-ripening" of the buds. There seems to be no correlation between dormancy and rooting, but there is a decided relationship between cool temperature and preparation of buds for growth. Plate LVI, figure 4, shows two holly plants made from cuttings taken on October 5, 1928, and



kept continuously in a warm house until photographed on April 10, 1929. In each case a lateral bud instead of the terminal bud gave rise to the new shoot. In similar cases terminal buds have lain dormant through the summer and then developed shoots after passing the winter in a cool place. Apparently the terminal buds have a more lasting rest period than lateral buds. Plate LVII, figure 5, illustrates the appearance of the *I. opaca* plants as new shoots and flower buds are developing. There is a difference between the pistillate and staminate buds, the former being solitary and the latter in clusters of two or more buds on one pedicel. To secure berries on potted plants it is necessary to propagate staminate plants so that they will flower concurrently with the pistillate plants. Insects do the pollination work if flowering occurs in the spring, but if grown in greenhouses during the winter the flowers must be pollinated by hand. Plate LVIII, figure 6, shows two holly plants that grew from cuttings taken January 5, 1927. They were potted on April 1, 1927, and photographed in November, 1927. They have a good quota of berries for small plants. The flower buds were present when the cuttings were made and were not disturbed through the process of propagation.

#### DISCUSSION

The percentage of rooting in different lots of holly (*Ilex opaca*) cuttings varied from zero to near 100 percent. Lots taken from different trees on the same day varied from 39.5 percent to 81.5 percent rooting. Material from Massachusetts varied slightly more than that from New Jersey. The difference might be due to inherent factors or to our failure to pick comparable cuttings. If the cuttings had been comparable no variation would have resulted, but it is physically impossible to pick from one tree 1000 cuttings which have had the same environment. Material collected for cuttings came from different parts of the tree, and therefore had not been subjected to the same external environment. These environmental factors might determine the difference in response, but until we know more about the underlying principles controlling root growth from cuttings such problems will remain unsolved.

Since holly trees from which cutting material was taken are of seedling origin, there may well be inherent differences between trees. They show different leaf characters, different rates of growth, and some difference in susceptibility to disease. This being true, it is easy to understand why lots from different trees might respond differently but the question still remains why carefully selected cuttings from the same tree show variation in response. To answer such questions we must know vastly more than at present about the secrets of plant growth. Probably there are a number of factors operating to initiate roots. When all conditions are satisfied, roots begin to grow, though a variation of any one factor might change the type of response. Some roots are slow to appear though they have been initiated. In such cases possibly nutrition plays a rôle.

The results showing seasonal effects on vegetative propagation of holly are definite to the extent that succulent material is worthless and that after January 1 the rooting response is apt to be poor. The succulent material rots easily after being placed in the medium while the cuttings taken late are likely to have their leaves injured by cold weather. This probably would not hold for trees growing in the south where no winter injury occurs. During January 1927, Maryland material responded as well as that taken earlier in the winter.

As mentioned above, succulent material does not hold up but as it matures toward the middle of July it becomes favorable for cuttage. Results in table 1 indicate that the current-year growth alone is slightly better than when a piece of two-year-old stem remains attached. Old stems, however, retain their capacity to form roots for several years. So far the age limits have not been determined but five-year-old stems have been successfully used.

Media tests shown in table 1 are interesting in that they were conducted to include acid and alkaline material. Rooting has occurred in media showing a pH value of 3.5 to 8. Best results have been obtained around pH 5, but some rooting occurred at pH 8. Most consistent results have occurred in a mixture by volume of 50 percent peat moss and 50 percent sand. Cuttings in pure peat moss have at times rooted well but they have not given consistent results, probably due to variable amounts of water held by this medium during the different trials. Neutral water-washed sand has not been so good as slightly acid bank sand. Here again it might be a question of water-holding capacity since the bank sand had more sediment and kept a more nearly constant water supply. Certainly the oxygen and CO<sub>2</sub> content of the air in the medium has not been a determining factor. The highest CO<sub>2</sub> content detected at any time was in pure peat moss where it reached 0.5 percent. Sand usually had 0.1 percent, and mixtures of peat moss and sand had from 0.1 to 0.3 percent depending upon when the readings were made. The media were four to five inches deep and had about the same amount of oxygen as would be found in the air of the house. The writers have shown that willow cuttings will root when oxygen is low; they will also root when carbon dioxide is high providing the oxygen content is approximately 20 percent. While we should not generalize from facts applying to willow, it is likely that holly roots can grow in fairly high amounts of CO<sub>2</sub> and much less oxygen than is normally found in air.

One or more leaves are needed for rooting evergreen holly cuttings. Deciduous varieties will root without leaves either in dark or light. The leaves of *Ilex opaca* have usually been classed as sclerophyllous, but we find that they lose water rapidly, and one of the hardest problems has been to keep them fresh while the cutting is in the rooting medium. The most successful method of planting has been to slant the stem so that the leaves rest directly on the medium. The nurserymen generally consider the

holly difficult to propagate. Probably their failures were due to their methods of planting. Shallow and vertical planting bring very poor results. If high humidity could be maintained the latter method might serve, but ventilation is necessary to hold the temperature down, and during this operation the cuttings are likely to wilt.

Diseased leaves are a source of trouble. The holly leaf-spot disease causes the leaves to fall from the cuttings before rooting can occur. Cutting material, therefore, should be selected from healthy trees.

If in good condition, leaves function to increase carbohydrates when in the medium. Microchemical tests have shown that more starch is present at the time roots are forming than when the cuttings were made. This indicates an important purpose of green tissue in light even for cuttings. There is no indication that the presence or absence of starch has been a limiting factor, for cuttings with a low starch supply have been known to root and some with abundance of starch have failed to root though they appeared to remain in good condition.

Artificial illumination for 6 hours in addition to normal sunlight has increased the percentage of rooting and has favored root growth of *Ilex crenata* and *I. glabra* (fig. 1). It has favored root growth of *I. opaca* (fig. 2) but has not always increased the percentage of rooting. When the electric lights are burning, the air over the cuttings is somewhat drier than normally and it is likely that some of the shallow planted cuttings suffered injury from drying. Other genera such as *Azalea*, *Andromeda*, and *Camellia* have given increased percentage of rooting and increased growth of roots with extra light. It is probable that *Ilex opaca* would respond like *I. crenata* in extra light if precautions were taken to keep the leaves in good condition throughout the experiment. At any rate, once roots are initiated there can be no doubt about the favorable effect of extra light on their growth.

The time required for roots to appear on holly cuttings varies with the temperature. This fact is best illustrated by the figures given for *Ilex verticillata* rooted under various constant temperatures. Rooting occurred in 18 days at 80.6° F., in 28 days at 68° F., and in 42 days at 59° F. This shows that a wide range of temperature can be used and that the effect will be only to vary the time of rooting. The evergreen varieties are more difficult to handle since both light and temperature must be considered. There is some indication that 68° to 78° F. is a favorable range, but no doubt higher or lower temperatures could be used successfully. At 68° to 75.2° F. rooting of *I. opaca* cuttings starts in 21 days. Approximately 75 percent of the cuttings root within 60 days. The root system is generally large and sufficiently hardened for potting after 90 days. Both *I. glabra* and *I. crenata* require less time than *I. opaca*. Though few experiments were carried out with *I. cornuta* and *I. aquifolium*, they appeared to have about the same time and temperature requirements for rooting as *I. opaca*.

The roots of *I. opaca* generally arise just above the cut end of the stem. Occasionally roots appear from the callus and in immature tissue they often arise along the stem some distance from the base. All the other varieties studied showed some variation but in general they are essentially like *I. opaca*.

The growth of *Ilex opaca* plants produced from cuttings was interesting in that the new shoots developed just about the same as they would have, had they remained on the mother tree. Winter buds with flower and shoot primordia mature in early fall. In nature these buds have a rest period of two to three months during which time they need a cold temperature to prepare them for growth in the spring. Cuttings taken into warm greenhouses in October will form roots regularly but new shoots do not develop normally if the plants are left continuously in a warm place. Figure 4 shows that laterals may grow at the expense of terminals when the plants do not have the advantage of a low temperature period. An occasional terminal will grow, but as shown by the data on another page, out of 180 plants resulting from cuttings taken in October and kept in a warm house only 17 had made shoot growth by April 9. Of 200 plants from cuttings taken on December 17 all were producing shoots on April 9. Another set of more than 2000 plants from cuttings taken in October made practically no growth during the following summer, but after remaining in a cold house for the winter they made supposedly normal growth in April, as shown by figure 5. The new shoots have flower buds and though a year late in developing they appear to be normal. If pollinated when ripe, the pistil develops into a green berry which matures and turns red after seven to eight months. Figure 6 shows two plants that flowered in May and produced berries which were red when photographed the following November. Their value as Christmas plants can be readily seen. The main problem in producing such plants is to select cuttings with flower primordia. Although there is some variation from year to year, the majority of terminal stems on trees in good light have flower primordia in the three or four buds nearest the tip. Both pistillate and staminate types should be propagated at the same time so as to insure a supply of pollen when the stigmas are ripe.

#### SUMMARY

1. Holly (*Ilex opaca*, *I. crenata*, *I. glabra*, *I. cornuta*, and *I. aquifolium*) was multiplied through vegetative propagation by means of cuttings. In the case of *Ilex opaca*, the percentage of rooting varied according to the source of cutting material. Of four lots taken at the same time from different trees in Massachusetts the percentage was approximately 40, 60, 80, and 80 percent, respectively. Even though three types of cuttings were made from each lot the percentage of rooting remained fairly consistent for each tree. Similar results were obtained with material from Maryland and New Jersey.

2. Rooting was equally good from August 1 to January 1. Results were generally unsatisfactory after January 15 because of frost injury.

3. Three types of cuttings were tried. Cuttings made of current year stems were slightly better than where old stems remained attached. A four- or five-inch cutting of current growth plus a portion of two-year-old stem gave satisfactory results. Culls or material four to six inches in length taken at random from stems two to five years old gave a greater variation and a lower percentage of rooting than other types.

4. Of the five different media used, a mixture of 50 percent sand with 50 percent peat moss by volume was generally the best. Rooting was obtained, however, over a pH range of 3.6 to 8 in the various media.

5. Cuttings of evergreen hollies did not root when all leaves had been removed. *Ilex verticillata*, a deciduous variety, rooted when no leaves were present.

6. *Ilex opaca* cuttings started rooting in 21 days, but to obtain a high percentage of rooting it was necessary to leave the material in the medium for 3 to 4 months.

7. Holly cuttings rooted at various temperatures from 59° to 80° F., but for practical purposes 65° to 75° F. was considered a satisfactory range.

8. Stems of *Ilex opaca* increased in starch and decreased in reducing substances while in the rooting medium. *Ilex crenata* cuttings kept in the dark lost starch regularly for 6 weeks and did not root. Comparable sets in light increased in starch and formed roots.

9. Dormant cuttings taken in October produced roots but very few shoots when kept continuously in a warm place. The buds did not develop until after being stored two or more months in a cool place. Many plants lay without top growth throughout the summer of 1928 but having been stored in a cool place the following winter, they made normal growth in the spring of 1929.

10. Potted holly plants (*I. opaca*) when properly selected and pollinated produced crops of berries during the first season.

## EXPLANATION OF PLATES

### PLATE LV

FIG. 1. *Ilex crenata* cuttings, Nov. 16, 1928, to Jan. 9, 1929. Top row, cuttings given 6 hours of extra light in addition to normal sunlight. Lower row, cuttings given normal sunlight only.

FIG. 2. *Ilex opaca* cuttings, Oct. 4, 1928, to Dec. 14, 1928. Top row, cuttings given full normal sunlight plus 6 hours of artificial illumination from 1,000-watt nitrogen bulbs. Lower row, cuttings given full sunlight only.

### PLATE LVI

FIG. 3. *Ilex opaca* cuttings, Dec. 17, 1927, to Feb. 20, 1928. The photograph shows one complete set of cuttings to illustrate variability in size of roots appearing after two months time.



ZIMMERMAN AND HITCHCOCK: HOLLY PROPAGATION

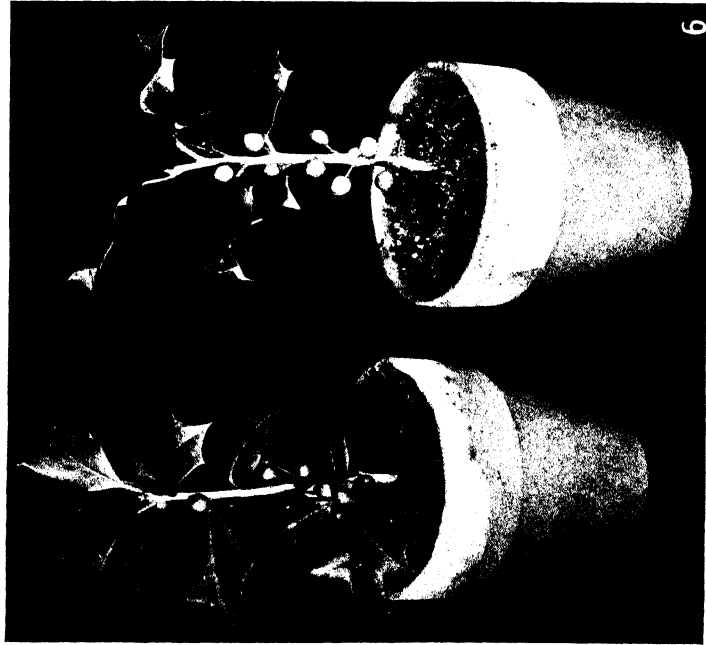




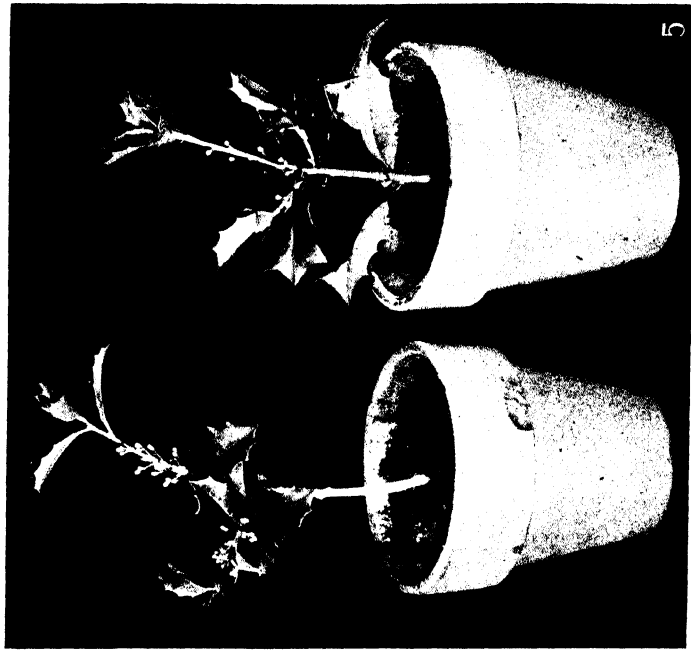
ZIMMERMAN AND HITCHCOCK: HOLLY PROPAGATION







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ZIMMERMAN AND HUTCHCOCK: HOLLY PROPAGATION



FIG. 4. *Ilex opaca* plants from cuttings, Oct. 5, 1928, to April 10, 1929. For explanation of figure see page 564 of the text. (Compare with figure 5.)

#### PLATE LVII

FIG. 5. *Ilex opaca* plants from cuttings, Oct. 28, 1927, to April 9, 1929. The picture illustrates development of shoots after the buds had lain dormant through an entire growing season. Previous to the summer of 1928 they were kept continuously in a warm house. During the winter of 1928 and 1929 they were stored in a cold greenhouse. As the weather became warm in the spring the new shoots appeared. The plant on the left is staminate and the one on the right is pistillate. (Compare with figure 6.)

FIG. 6. *Ilex opaca* plants from cuttings, Jan. 5, 1927, to Nov. 18, 1927. The buds had passed their rest period before the cuttings were made, consequently new growth appeared as soon as root systems were established. The flowers were pollinated in May and the resulting berries were red by December. (Compare with figure 5.)

## EFFECT OF ENVIRONMENTAL CONDITIONS ON THE CHLOROPLAST PIGMENTS

JOHN D. GUTHRIE

### INTRODUCTION

Prior to the work of Willstätter and his coworkers (1912) our knowledge of the chloroplast pigments was in an uncertain state. Willstätter succeeded in isolating these pigments in the pure condition and determined their chemical nature. He showed that they possess the following formulas:

Chlorophyll *a*  $[C_{32}H_{30}ON_4Mg](COOCH_3)(COOC_{20}H_{39})$ ,

Chlorophyll *b*  $[C_{32}H_{28}O_2N_4Mg](COOCH_3)(COOC_{20}H_{39})$ ,

Carotin  $C_{40}H_{56}$ ,

Xanthophyll  $C_{40}H_{56}O_2$ .

These formulas seemed to indicate an interconversion of the pigments in the process of photosynthesis. Therefore, Willstätter devised methods for the quantitative determination of the pigments in leaves. Applying these methods to leaves gathered from various natural habitats, it was found that the ratio of chlorophyll *a* to chlorophyll *b*, the *a/b* ratio, and the ratio of carotin to xanthophyll, the *c/x* ratio, were practically constant. No diurnal change in these ratios could be detected. For these reasons he abandoned the idea of the interconversion of the pigments. Nevertheless, the formulas are so suggestive of a relationship between the pigments that theories based on such hypothetical relationships are advanced from time to time. Usually no attempt is made to substantiate such speculations by experimental work, and often the known facts are neglected.

The main purpose of the work reported here was to provide additional acts that might bear on such theories. Equipment that placed environmental factors more or less under the control of the experimenter was available. Using this equipment, conditions differing greatly from those to be found in nature could be produced. The effects of light duration, light quality, light intensity, carbon dioxide, and mineral nutrients were studied. A few analyses were made on mosaic tomatoes. In general the results show the constancy of the pigment ratios, but they also show that under certain conditions these ratios are changed.

Aside from the main object, other problems presented themselves during the course of the investigation. A new quantitative method for fraction-

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ating chlorophyll *a* and chlorophyll *b* was devised; a series of substitute standards for chlorophyll was prepared; and a brown pigment that could be extracted from leaves with dilute acetone was studied.

### HISTORICAL

Sorby (1873) concluded, from spectrophotometric examination of leaf extracts, that the ratio of chlorophyll *a* to chlorophyll *b* ranges from 5.9 : 1 to 7.7 : 1, and that this variation probably depends on the length of time the leaves are exposed to the sun. Tswett (1907), using a spectrographic and also his chromatographic method, concluded that the *a/b* ratio varies from 4 : 1 to 6 : 1. Jacobson and Marchelewski (1912) come to the conclusion that the *a/b* ratio varied with environmental conditions. They precipitated the chlorophyll as phäophytin from leaf extracts, and then examined it spectrographically. Their chief error, and no doubt the cause of the fluctuations observed, was a partial fractionation of the pigments, which has been shown by Willstätter to occur in this precipitation, due to phäophytin *a* being more soluble than phäophytin *b*. More recently Wlodek (1921) came to conclusions similar to those of Jacobson and Marchelewski based on the spectroscopic examination of living leaves. He measured the width of the first absorption bands of the pigments and found them to vary. As a matter of fact, these bands overlap, so it is not possible to measure their width. It is very doubtful if the fluctuations he observed had anything to do with the *a/b* ratio.

In a study of autumnal changes, Willstätter (1918) found that there was often a conversion of carotin into a pigment that at least behaved like xanthophyll in the analysis. There was little change in total carotinoids. An increase in water-soluble brown pigment was noted. Goerrig (1918) working on carotinoids in autumnal coloration also noted little change in total carotinoids, but an increase in water-soluble brown pigment. Tswett (1908) also was of the opinion that autumnal coloration was due to water-soluble brown pigments, and found the carotinoids present were not the ones normally present in leaves.

Willstätter (1918) studied the effect of high carbon dioxide and high light intensity on detached leaves. He found that the ratio of carotin to xanthophyll shifts to a lower value under these conditions. There was also a small lowering of the *a/b* ratio.

### METHODS

In general the methods used were based on the work of Willstätter and Stoll. They were not of a high degree of precision, but were the best available. Two methods for fractionating chlorophyll *a* and *b* were used throughout the work. One was the method of Willstätter, modified only in minor details, and the other a new method worked out by the author. It was thought best to use both methods in order to avoid misleading results

that might arise should one contain a systematic error. Besides, this made possible the comparison of the new method with the more established one under a wide range of conditions. During part of the work total chlorophyll was determined by a method that was essentially that of Willstätter. More will be said later concerning the results of comparing these three methods and the errors involved in the determinations.

The carotinoids were determined by the method of Willstätter, using a Bausch and Lomb colorimeter for comparison against potassium dichromate. In all colorimetric comparisons, several readings were taken with the standard on the left, then an equal number with the standard on the right, and all values averaged. Six readings were usually made in the chlorophyll determinations, ten in the carotinoid determinations. During the last part of the work a spectrophotometer was available, and the carotinoids were determined as recommended by Schertz (1923).

### **Willstätter's Methods for the Four Chloroplast Pigments**

Briefly these methods are based on the following principles:

(1) The quantitative extraction of the pigments with acetone and their quantitative transfer to ether.

(2) Conversion of the chlorophylls to phäophytin by acidification; saponification of the phäophytin with hot, concentrated, methyl alcoholic potassium hydroxid to phytochlorin *e* and phytorhodin *g*; and fractionation of these derivatives from ether solution with 0.835 and 3.47 normal hydrochloric acid.

(3) The separation of the chlorophylls from the carotinoids by saponification in the cold with methyl alcoholic potassium hydroxid, and the fractionation of carotin and xanthophyll between petroleum ether and methyl alcohol.

For the details of these methods the reader is referred to Willstätter and Stoll (1912).

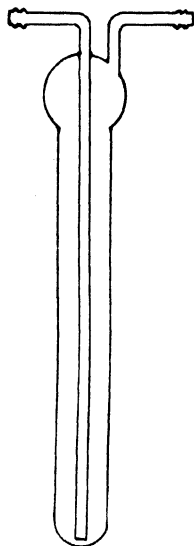
### **A New Method for Chlorophyll a and Chlorophyll b**

The principle on which this method is based is the methylation of the chlorophyll in the leaf by refluxing with methyl alcoholic hydrochloric acid, quantitative extraction being accomplished by the same procedure. The resulting methyl phäophorbids are then fractionated from their ether solution with 5.05 and 6.75 normal hydrochloric acid. The method follows:

Place 30 g. of leaves in an 800-cc. Kjeldahl flask and add 200 cc. of 0.6*N* methyl alcoholic HCl. Put a condenser, of the type shown in text figure 1, in the neck of the flask and reflux on the steam bath for one hour. Pour the methyl alcoholic HCl from the leaves into a beaker, add 100 cc. more to the leaves, reflux 10 minutes, and pour off. Do this three times. Combine the extracts, cool, and filter through a rapid paper.

Place the extract in a 4-liter separatory funnel and mix with 350 cc. of ether. Add about 1½ liters of water slowly. The ether separates as a

layer at the top. This ether layer contains most of the chlorophyll in the form of methyl phäophorbid. Wash the aqueous layer with 75 cc. of ether in order to remove the last traces of the chlorophyll derivatives. Do this by shaking vigorously with the ether and waiting until the ether separates to the top. Combine this wash ether with the main bulk. The aqueous layer often remains brown, but this is due to pigments other than chlorophyll, which are not soluble in ether. At this point the ether may be placed in the ice box over night without appreciable loss of pigments.



TEXT FIG. 1. Condenser used in new method for chlorophyll determinations.

Place the ether in a 500-cc. separatory funnel and extract three times with 25-cc. portions of HCl, sp. g. 1.125, and twice with 15-cc. portions of HCl, sp. g. 1.19. Make these extractions by shaking the ether vigorously with the acid and then allowing the layers to separate. Run the extracts into a second 500-cc. separatory funnel. Add 150 cc. of ether, and transfer the pigments to it by slowly diluting with water and shaking vigorously. This is another point at which the extracts may be kept in the ice box over night.

The pigments are ready for fractionation. Add 50 cc. of 5.05*N* HCl and shake vigorously. Place this extract in a second separatory funnel, add 20 cc. of ether, shake vigorously, and when the layers separate run the extract into a 500-cc. volumetric flask. Add the wash ether back to the main bulk. Repeat this process until the flask is near the mark. Make to volume with the same acid and mix by pouring out into a dry beaker and back into the flask. The flask contains the chlorophyll *a* of the sample in the form of



methyl phäophorbid *a*. Filter a portion of this through a rapid paper and compare at once with the standard for the *a* fraction. Extract the ether with 100-cc. portions of 6.75*N* HCl. Run these extracts into a 500-cc. volumetric flask, bring to volume, mix, filter, and compare with the standard for the *b* derivative.

The standards may be prepared by fractionating a known mixture of methyl phäophorbids *a* and *b*, similar in amount and ratio to that expected in the sample, or with the substitute standards described further on. The methyl phäophorbids were prepared and purified as described by Willstätter (1912).

### Method for Total Chlorophyll

The method used for total chlorophyll in some of the analyses was essentially that of Willstätter. The alkaline chlorophyll extracts from the carotinoid determinations were made up to a convenient volume, usually one liter, and compared with a standard made by saponifying a known weight of chlorophyll in a manner identical with the unknown, or with a substitute standard described in a previous paper (1928).

### The Pigment Soluble in Dilute Acetone

In Willstätter's method a preliminary extraction was made with 30-percent acetone. This extract was brown in color. In Willstätter's work it was discarded. He mentions, however, that it varied in intensity from plant to plant and with conditions of growth. Although the nature of the pigment or pigments present in this extract was unknown, it was thought desirable to make some study of it. The extracts were made to volume, allowed to stand until colloidal material flocculated, and then filtered. Relative amounts were determined by colorimetric comparison.

### Substitute Standards

Throughout the work substitute standards were used for most of the determinations. They proved superior to the regular standards due to ease of preparation, stability, and capacity for exact duplication. Furthermore, they should be of value to those not desiring to go to the trouble of preparing pure compounds for making the regular standards.

That these substitutes give results essentially the same as the regular standards is shown by the following values obtained by applying both substitute and regular standards to the same series of samples:

Chlorophyll		Chlorophyll <i>b</i>		<i>a/b</i> Ratio	
Standard	Substitute	Standard	Substitute	Standard	Substitute
0.537	0.535	0.196	0.195	2.78	2.78
0.625	0.597	0.227	0.220	2.80	2.75
0.625	0.596	0.226	0.219	2.80	2.75
0.073	0.068	0.028	0.027	2.65	2.56

The substitute standards for chlorophyll *a* and *b* were standardized by fractionating known mixtures of the methyl phäophorbids *a* and *b* in a manner identical to that given in the above methods.

## STOCK SOLUTIONS

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ .....	10 g. per liter
$\text{K}_2\text{Cr}_2\text{O}_7$ .....	2 g. per liter
$\text{NH}_4\text{OH}$ .....	twice normal

To make up a standard, measure the indicated number of cc. of  $\text{CuSO}_4$  and  $\text{K}_2\text{Cr}_2\text{O}_7$  into a 100-cc. volumetric flask, add 10 cc. of 2*N*  $\text{NH}_4\text{OH}$ , make to volume, and mix.

In the following notations the first number indicates how many cc. of  $\text{CuSO}_4$  to use, and the second indicates the number of cc. of  $\text{K}_2\text{Cr}_2\text{O}_7$ . 10 cc. of 2*N*  $\text{NH}_4\text{OH}$  are used in each case. The substitutes when set at 20, 30, or 40 mm. are equivalent to the milligrams of chlorophyll *a* or *b* as indicated; this of course, in a volume of 500 cc., which is the same as that to which the unknowns are brought.

	Cc. $\text{CuSO}_4$	Cc. $\text{K}_2\text{Cr}_2\text{O}_7$	
Willstätters method	No. 1.....40	20 $\approx$ 55.7 mg. chlorophyll <i>a</i>	
	14.5	30 $\approx$ 18.5 mg. chlorophyll <i>b</i>	
	No. 2.....25.5	17 $\approx$ 38.8 mg. chlorophyll <i>a</i>	
	10.5	22 $\approx$ 13.3 mg. chlorophyll <i>b</i>	
	No. 3.....19	14 $\approx$ 27.7 mg. chlorophyll <i>a</i>	
	6.5	19 $\approx$ 9.5 mg. chlorophyll <i>b</i>	
New method	No. 1.....40	23 $\approx$ 54.1 mg. chlorophyll <i>a</i>	
	19	38 $\approx$ 18.6 mg. chlorophyll <i>b</i>	
	No. 2.....25.5	20 $\approx$ 41.6 mg. chlorophyll <i>a</i>	
	14.5	30 $\approx$ 13.3 mg. chlorophyll <i>b</i>	
	No. 3.....15.5	14 $\approx$ 27.0 mg. chlorophyll <i>a</i>	
	10.5	20 $\approx$ 8.8 mg. chlorophyll <i>b</i>	

Potassium dichromate, 2 g. per liter, was used as a substitute for true solutions of the carotinoids. The values for this standard obtained by author do not agree with those given by Willstätter, perhaps due to a difference in color vision. For this reason it is recommended that workers establish their own values for this substitute whenever possible, unless they are interested only in relative results.

The values used in this work follow: Willstätter's values are given in parentheses:

$\text{K}_2\text{Cr}_2\text{O}_7$ 2 g. per Liter Set at:	Carotin 26.8 mg. per Liter Matches at:
19 mm.....	25.0 mm. (25.0)
27 mm.....	34.5 mm.
35 mm.....	41.0 mm.
41 mm.....	45.5 mm. (50.0)

K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> g. per Liter Set at:	Xanthophyll 28.4 mg. per Liter Matches at:
14 mm.....	22.8 mm. (25.0)
19 mm.....	27.5 mm.
27 mm.....	36.6 mm. (50.0)
35 mm.....	43.0 mm.

As a primary standard for xanthophyll a solution standardized spectrophotometrically by the method of Schertz was used. The reason for this was the lack of satisfactory material from which to prepare xanthophyll. A small preparation from soy bean leaves gave a specific transmissive index of 1.87 in ether, considerably lower than the value 2.09 given by Schertz (1925). A solution of pure carotin was used as a primary standard against which to compare the potassium dichromate. This was prepared by a method different from that described by Willstätter.

### Preparation of Pure Carotin

6.5 kg. of fresh carrots were ground through a nixtamal mill into acetone and then extracted with acetone on an asbestos suction mat. The first two extracts, due to dilution of the acetone with water in the carrots, contained little carotin and were discarded. The next three extracts contained the bulk of the pigment. These were combined and diluted slowly with about one-third of their volume of water. Carotin crystallized on standing in an almost pure state, as shown by the high transmissive index (1.97) of solutions of the preparation. The crystals were filtered off with suction after the extracts had stood over night. The yield was 230 mg. The carotin was purified by recrystallization from petroleum ether. This increased the specific transmissive index to 2.11 for the mercury line 435.8. Further recrystallization did not change this value. The melting point was 169° C., which agrees with that originally given by Willstätter and Mieg (1907) rather than the more recent value (174°) given by Willstätter and Stoll and also found by Schertz (1925). Schertz gives 1.915 for the specific transmissive index of carotin in petroleum ether. He prepared his carotin from dried carrots. On the basis of its higher melting point, his preparation might be expected to be purer than the one described here, yet the specific transmissive index, which Schertz advances as a criterion of purity, shows the opposite to be true.

### Probable Error of Methods

On account of the impossibility of running a large number of determinations at one time, the probable error of the methods could not be found in the usual manner. The value of the probable error was estimated from the results of duplicate determinations in the following manner:<sup>2</sup>

<sup>2</sup> I am indebted to Doctor Frank Wilcoxon for suggesting this method of determining the probable error.

Find the deviation of the duplicates from each other in percent of their mean. Take the sum of the squares of these deviations. Divide by the number of duplicates and take the square root. Multiply by  $.67/\sqrt{2}$ . This is equivalent to the following:

$$\text{Probable error singular} = \frac{.67}{\sqrt{2}} \sqrt{\frac{\sum d^2}{N}}.$$

Applying this to the data at hand it was found that for Willstätter's method for chlorophyll *a* and *b* the probable error singular for total chlorophyll was 1.6 percent, for the *a/b* ratio 3.7 percent. With the new method for total chlorophyll *a* and *b* the probable error singular for total chlorophyll was 4.1 percent, for the *a/b* ratio 3.5 percent. With Willstätter's method for the carotinoids the probable error singular for total carotinoids was 2.6 percent, for the *c/x* ratio 4.7 percent.

Since working out the above method it was found that a method essentially the same, and giving the same results when applied to the data at hand, has been published by Fleisch (1926).

### A Comparison of Methods

Three methods giving total chlorophyll values were used throughout the work. In many cases these were used on identical material. A comparison of values so obtained showed that Willstätter's method for total chlorophyll gave values averaging 43 percent higher than his method for chlorophyll *a* and *b*. The new method gave values averaging 50 percent higher than those by Willstätter's method for chlorophyll *a* and *b*. The *a/b* ratios averaged 20 percent higher by the new method than by Willstätter's.

The low values with Willstätter's method were probably due to destruction of chlorophyll during the saponification with hot concentrated potassium hydroxid, this destruction being associated with the presence of impurities and therefore not occurring when the standard was saponified. Willstätter mentions the possibility of a loss in saponification, but considers it negligible. Unfortunately no comparison of his two methods can be found in his work.

In tables 7, 9, and 11 values are given by both the colorimetric and spectrophotometric methods for the carotinoids. It will be seen that although they differ somewhat in a few cases, the conclusions can be drawn from the data by either method.

### Preservation of the Sample

All the analyses in this work were made on fresh material, immediately after gathering. With a view toward future work the possibility of storing the samples in a frozen condition was investigated. The results are summarized in table 1. The decrease in total chlorophyll is almost negligible, but there is plainly a shift in the *a/b* ratio. Odds that this difference is

significant are 990 : 1 by "Student's" method (see Love, 1924). This seems to be due to the conversion of a small amount of chlorophyll *a* to chlorophyll *b*. The odds are 85 : 1 that there is a decrease in *a*, and 45 : 1 that there is an increase in *b*.

TABLE 1. *Chlorophyll Analyses on Samples Preserved by Keeping Frozen Compared with Analyses on the Fresh Tissue*

Plant	Time Stored	Method Used	Chlorophyll <i>a</i> in percent of Fresh Weight		Chlorophyll <i>b</i> in percent of Fresh Weight		Total Chloro- phyll in percent of Fresh Weight		<i>a/b</i> Ratio	
			Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
Sunflower..	7 days	New	0.119	0.113	.042	.044	0.161	0.157	2.9	2.6
" ..	10 "	"	0.143	0.133	.033	.041	0.176	0.174	4.5	3.3
" ..	31 "	"	0.137	0.111	.038	.043	0.175	0.154	3.8	2.6
Tomato....	4 months	Willstätter's	0.100	0.086	.024	.026	0.124	0.112	4.2	3.4
" ..	2 "		0.039	0.036	.017	.020	0.056	0.056	2.4	1.8
" ..	28 "		0.080	0.074	.031	.036	0.111	0.110	2.7	2.1

Samples showed a marked loss in total chlorophyll when dried and stored. It may be that by exercising care serious losses can be avoided by the use of this method, but it cannot be recommended as generally applicable.

#### RESULTS OF ANALYSES OF PLANTS GROWN UNDER DIFFERENT CONDITIONS

In reporting the results of these experiments, the values for the dry weight determinations are omitted from the tables in order to save space. For the same reason only total chlorophyll or total carotinoid percentages and the *a/b* or *c/x* ratios are tabulated, except where there is a marked shift in the ratios. In these cases the pigments are recorded separately. In some instances the figures represent averages of several determinations. However, the interpretation of the results is based on the individual values.

One should not attach much significance to small differences occurring vertically in the tables, owing to the somewhat empirical nature of the methods and the different ages of the plants. However, the values given horizontally in the tables are results of analyses run at the same time, under the same conditions, and on plants of the same age. They are therefore comparable. In studying the data the probable errors of the methods are of value in deciding on the significance of differences. However, the data are best adapted to the use of "Student's" method. The odds have been worked out by this method wherever the data are sufficient. These odds are given in the discussion of the results of each experiment.

#### Experiment 1. Effect of Increased Carbon Dioxid

This experiment was started on November 16, 1925, and ran through the winter. The object was to see what effect carbon dioxid would have on plants. In general they responded with increased growth, even though light

TABLE 2. *Analyses of Plants Grown in Various Concentrations of Carbon Dioxide*

Plant	Substance Determined	Method Used	Total Pigment in percent of Fresh Weight				Total Pigment in percent of Dry Weight				Ratio, Either a/b or c/x			
			Control	Flue Gas	0.3 per-cent	1.0 per-cent	Control	Flue Gas	0.3 per-cent	1.0 per-cent	Control	Flue Gas	0.3 per-cent	1.0 per-cent
Tomato.....	Chlorophyll*	Willstätter's	0.133	0.133	0.138	0.140	1.36	1.38	1.11	1.17	3.1	3.0	3.1	3.2
".....	"†	New	0.149	0.134	0.148	1.68	1.56	1.31	1.18	1.36	4.3	4.0	4.0	4.1
Seed leaves, soy bean..	"	Willstätter's	0.130	0.162	0.152	0.158	0.84	0.94	0.86	0.89	3.6	3.4	3.3	4.3
Soy bean.....	"	"	0.165	—	0.162	0.162	0.55	—	0.58	0.55	2.6	—	2.9	2.4
" ".....	"	New	0.213	0.206	0.213	0.218	0.76	0.68	0.76	0.70	3.7	3.1	3.3	3.6
Sunflower....	"	"	0.178	—	—	0.168	—	—	—	—	4.2	—	—	3.6
Tomato.....	Carotinoids	Willstätter's	0.0136	0.0127	0.0116	0.0124	0.165	0.130	0.113	0.118	0.57	0.61	0.55	0.50

\* Average of three analyses.

† Average of four analyses.

intensity was poor during most of the experiment. Owing to necessary temperature control, it was hard to regulate the carbon dioxide concentration. Analyses for carbon dioxide were made daily and in general the values were kept close to those desired. The temperature in all houses was 25° C. The conditions were as follows:

- (1) Control. Ordinary greenhouse conditions.
- (2) Furnace gas. Purified flue gas from boilers. Carbon dioxide content of air kept near 0.3 percent.
- (3) 0.3-percent house. Carbon dioxide kept near 0.3 percent by means of gas from tanks.
- (4) 1.0-percent house. Carbon dioxide kept near 1.0 percent by means of gas from tanks.

The results are summarized in table 2. Aside from carbon dioxide increasing the chlorophyll in the seedling soy beans, there is little effect on the green weight basis. Data given later will show that seedlings or young plants tend to respond with increased chlorophyll production under conditions favoring rapid photosynthesis, although the effect may be in the opposite direction as the plants grow older. This effect is probably due to the young plants having an abundant supply of all other materials necessary for chlorophyll production except carbohydrates. Later in the life of the plant these are exhausted, and an increase in chlorophyll production is no longer noted.

Considering the dry weight percentages, no significance can be attached to any chlorophyll differences in the soy beans. With the tomatoes, however, a marked reduction of chlorophyll is evident. Applying "Student's" method to the individual determinations, the odds for the 0.3-percent and 1.0-percent houses are 4,000 : 1 and 900 : 1, respectively. The difference of the dry weight results from the green weight values can be accounted for by an increase in the percentage of dry matter due to extra carbon dioxide, no doubt due to increased carbohydrate production.

There is a little evidence of a tendency for carbon dioxide slightly to decrease the  $a/b$  ratio. The odds are 8 : 1, 8 : 1, and 2 : 1 for the furnace gas house, 0.3-percent house, and 1.0-percent house, respectively. The differences are so small that they may be due to some constant error in the methods. In any case it is best for the present to attach no significance to them.

The data on the carotinoids are meager. However, the carotinoids are low in the houses with extra carbon dioxide when expressed on the dry weight basis. It is interesting to note that they should behave so similarly to chlorophyll toward carbon dioxide. This tendency for the carotinoids and chlorophyll to be similarly affected will also be noted in other experiments. The  $c/x$  ratio appears to remain unchanged.

## Experiment 2. Effect of Increased Day Length and Extra Carbon Dioxid. Eighteen-hour Day Series

The purpose of this experiment was to study the effect of increasing the length of day, with and without extra carbon dioxid. A gantry crane carrying 48 1,000-watt lights was used. This was moved over the greenhouses at night. At the start it gave an illumination of 723 foot candles, and at the close 450 foot candles as measured by a Macbeth illuminometer. Pyrliometer measurements were 0.32 and 0.30 gram calories per sq. cm. per minute, respectively. Plants were also given continuous artificial illumination in the constant light room. Here the light intensity started at 1,320 foot candles and closed at 1,100 foot candles. The pyrliometer values were 0.35 and 0.19. Carbon dioxid was supplied from tanks. The temperature under all conditions was 20° C. The experiment started February 24, 1926.

The conditions were as follows:

- (1) Control. Ordinary greenhouse conditions.
- (2) 18-hour day. Extra light from 12 P.M. to 6 A.M. with the gantry crane.
- (3) 18-hour day plus CO<sub>2</sub>. Extra light from 6 P.M. to 12 P.M. with the gantry crane. Carbon dioxid about 0.3 percent.
- (4) Continuous artificial illumination in the constant light room with carbon dioxid about 0.3 percent.

The results are summarized in table 3. Total chlorophyll on the green weight basis does not show large differences. On the average the values for the 18-hour day are slightly lower. The reduction of chlorophyll in the tomatoes was associated with a mottling effect. Taking the values for all the plants the odds are only 5 : 1 that the values in the 18-hour day series are significantly less. With the 18-hour day plus carbon dioxid the difference is greater. The odds here that the reduction observed is significant are 60 : 1. Also the values obtained in the constant light room are lower than in the control.

On the dry weight basis the chlorophyll values are distinctly lower with the 18-hour day and still lower with extra carbon dioxid. The odds are 88 : 1 and 1,666 : 1, respectively. Also, the odds that the 18-hour day plus carbon dioxid is lower than the 18-hour day without carbon dioxid are 184 : 1. Here we have increased photosynthesis acting to increase the dry matter without corresponding increase in chlorophyll. There is also the possibility of an increase in the rate of decomposition of chlorophyll under these conditions. The brown pigment shows a distinct increase.

The *a/b* ratio in the 18-hour day plus carbon dioxid tends to average slightly lower than the 18-hour day without carbon dioxid. The odds are only 4 : 1 and little importance can be assigned to the differences as a constant error may be involved.



TABLE 3. *Analyses in Day Length and Carbon Dioxide Experiment. Eighteen-hour Day Series*

Plant	Substance Determined	Method Used	Total Pigment in percent of Fresh Weight				Total Pigment in percent of Dry Weight				Ratio, Either a/b or c/x			
			Control	18-hr Day	18-hr Day + CO <sub>2</sub>	Cont. Illumi. + CO <sub>2</sub>	Control	18-hr Day	18-hr Day + CO <sub>2</sub>	Cont. Illumi. + CO <sub>2</sub>	Control	18-hr Day	18-hr Day + CO <sub>2</sub>	Cont. Illumi. + CO <sub>2</sub>
Sunflower...	Chlorophyll	New	0.133	0.125	0.124	0.121	1.08	1.02	1.03	0.86	3.1	3.7	3.3	3.2
"	"	Willstätter's	0.087	0.100	0.081	0.067	0.71	0.61	0.47	0.39	3.0	3.1	2.7	2.6
Tomato...	"	* New	0.145	0.131	0.124		1.29	1.08	0.85		3.5	3.3	3.3	
"	"	Willstätter's	0.138	0.085	0.111		1.28	0.81	0.67		2.8	2.5	2.7	
Soy bean...	"	"	0.182	0.163	0.139		0.86	0.70	0.45		2.5	2.4	2.2	
"	"	* New	0.255	0.284	0.168		1.29	1.08	0.56		2.5	2.5	2.3	
Sunflower...	Carotenoids	Willstätter's	0.0117	0.0089	0.0088	0.0081	0.087	0.044	0.049	0.051	0.40	0.46	0.47	0.33
Soy bean...	"	"	0.0194	0.0185	0.0080		0.086	0.068	0.027		0.45	0.44	0.48	
Sunflower...	Brown pigment †		1.00		4.12	8.16								
Tomato...	"	"	1.00	0.96	1.21									
Soy bean...	"	"	1.00	1.40	1.37									

\* Average of three analyses.

† Relative values. Control used as standard.

The carotinoids show changes essentially the same as chlorophyll, decreasing with extra carbon dioxid and increased day length. The  $c/x$  ratio shows no significant change.

### Experiment 3. Effect of Increased Day Length and Extra Carbon Dioxid. Twenty-four-hour Day Series

In this experiment the equipment was the same as in experiment 2. The temperature was kept at 25° C. Three white flame carbon arc lights were used in the constant light room in addition to the usual incandescent lights. The gantry crane started at 761 foot candles and ended at 765 foot candles. Pyrhelimeter readings were 0.36 and 0.31 gram calories per sq. cm. per minute, respectively. The constant light room started at 1450 and 0.24 and ended at 975 and 0.20. The experiment was started on January 28, 1927.

The conditions were as follows:

- (1) Control. Ordinary greenhouse conditions.
- (2) 24-hour day plus CO<sub>2</sub>. Extra light from the gantry crane 6 P.M. to 6 A.M. Carbon dioxid kept at about 0.3 percent by means of gas from tanks.
- (3) Continuous artificial illumination in the constant light room. Carbon dioxid about 0.3 percent.

The results of analyses are summarized in table 4. On the green-weight basis the effect of age of the plants is so evident that no treatment of the results as a whole can be made. With the seedling soy beans there is a marked increase in chlorophyll with extra carbon dioxid and long day. This effect of increased photosynthesis acting to increase chlorophyll in seedlings was noted in the carbon dioxid experiment and discussed there. This difference becomes less and less as the plants grow older, until finally in old age the long day, extra carbon dioxid plants contain distinctly less chlorophyll than the controls. This age difference is also evident in the sunflowers.

On the dry weight basis the seedling soy beans still show a higher chlorophyll percentage with extra carbon dioxid. But aside from these, the soy bean analyses show a marked decrease with extra carbon dioxid and increased day length, as is to be expected on the basis of the previous experiment. The odds are 770 : 1. The  $a/b$  ratio again shows a slight tendency to be lower with the extra carbon dioxid plants. The odds are 12 : 1.

The total carotinoids follow rather closely the total chlorophyll values. The  $c/x$  ratio tends to be slightly lower in the 24-hour day plus carbon dioxid plants.

Although the extracts containing the brown pigment were not compared colorimetrically, it was noted that those from the long day plus carbon dioxid plants were distinctly darker than those from the controls.

Tomatoes under the 24-hour day with extra carbon dioxid showed

TABLE 4. *Analyses in Day Length and Carbon Dioxide Experiment. Twenty-four-hour Day Series*

Plant	Days Under Exper.	Substance Determined	Method Used	Total Pigment in percent of Fresh Weight		Total Pigment in percent of Dry Weight		Ratio, Either a/b or c/z		
				Control	24-hr. Day + CO <sub>2</sub> *	Control	24-hr. Day + CO <sub>2</sub> *	Control	24-hr. Day + CO <sub>2</sub> *	24-hr. Day + CO <sub>2</sub> †
Soy bean.....	20	Chlorophyll	New	0.220	0.330	1.19	1.51	3.7	3.6	
" ".....	33	"	"	0.219	0.247	1.16	0.83	3.6	3.6	
" ".....	58	"	"	0.306	0.326	1.72	1.17	4.3	4.1	
" ".....	80	"	"	0.299	0.150	1.32	0.45	4.2	3.6	
Sunflower.....	35	"	Willstätter's	0.105	0.112	0.74	0.78	2.9	2.8	2.8
" ".....	70	"	New	0.176	0.159	1.28	0.82	4.4	4.2	
Soy bean.....	20	Carotinoids	Willstätter's	0.0245	0.0276	0.163	0.150	0.66	0.51	
" ".....	26	"	"	0.0238	0.0253	0.143	0.099	0.42	0.39	
Sunflower.....	35	"	"	0.0187	0.0150	0.133	0.104	0.47	0.42	0.42

\* Twelve hours natural, twelve hours artificial illumination.

† No daylight, continuous artificial illumination.

distinct injury, the plants making little growth. This injury is also characteristic of tomatoes in the constant light room. The effect on the pigments is described in the report on the next experiment.

#### Experiment 4. The Effect of Continuous Illumination on Tomatoes

It was found that when tomatoes were exposed to continuous artificial illumination in the constant light room, the leaves yellowed and later showed necrotic areas. For this experiment the plants were grown under ordinary greenhouse conditions, and then placed under continuous illumination. After several days analyses were made on these plants and on those left in the greenhouse as controls.

The results are shown in table 5. It was of course evident without

TABLE 5. *Analyses of Tomatoes Subjected to Continuous Illumination Compared with Plants Left under Ordinary Greenhouse Conditions*

Substance Determined	Method Used	Total Pigment in percent of Fresh Weight		Total Pigment in percent of Dry Weight		Ratio, either $a/b$ or $c/x$	
		Control	Continuous Illumination	Control	Continuous Illumination	Control	Continuous Illumination
Chlorophyll *	Willstätter's	0.085	0.055	0.59	0.25	2.4	2.1
"	"	0.069	0.039	0.51	0.21	2.2	1.9
"	New	0.124	0.068	0.99	0.35	4.2	3.8
Carotinoids *	Willstätter's	0.0104	0.0060	0.067	0.027	0.51	0.38
Brown pigment *		1.00	1.63				

\* Average of 4 analyses.

† Plants under continuous illumination had no extra carbon dioxide.

analysis that a decrease in chlorophyll had taken place. The data show this plainly. The decrease on the dry-weight basis is greater, due to a very large increase in carbohydrates in the continuously illuminated plants.

The  $a/b$  ratio shows a consistent lowering with continuous illumination. The odds are 1,000 : 1 that this is significant. The individual values for chlorophyll  $a$  and  $b$  (not given in table) show that both decrease under the effect of the light, but chlorophyll  $a$  decreases faster than  $b$ .

The carotinoids also show a distinct decrease under the action of the light. The  $c/x$  ratio is also lowered. Odds are 33 : 1 that this difference is significant. Values given individually for carotin and xanthophyll show that both decrease, the shift in the ratio being due to the more rapid decomposition of carotin. The brown pigment shows a large increase with continuous illumination.

#### Experiment 5. Effect of Light of Various Qualities

In this experiment greenhouses equipped with various glasses cutting out certain regions of the spectrum were used. No attempt was made to

TABLE 6. Analyses in Colored Glass Experiment Where Total Intensity is the Same in  $H_1$ ,  $H_2$ ,  $H_3$ , and  $H_4$ , but 20 percent lower in  $H_5$

[illegible]

keep the temperature constant, but it was maintained approximately the same in all the houses by forced ventilation. The equipment has been described by Popp (1926) and the reader is referred to his paper for further details.

Three series were run. In the first the light was kept approximately the same in houses 1, 2, 3, and 4 with shading cloth. The intensity in house 5 was about 80 percent of the others. The conditions were as follows:

H<sub>1</sub>. Ordinary greenhouse glass.

H<sub>2</sub>. A glass transmitting about 80 percent of the incident radiation at 290 m $\mu$ , the extreme limit for solar radiation.

H<sub>3</sub>. A glass cutting out the ultra violet.

H<sub>4</sub>. A yellow glass cutting out all wave lengths shorter than 472 m $\mu$ .

H<sub>5</sub>. An orange glass cutting out all wave lengths shorter than 526 m $\mu$ .

The results of the analyses are given in table 6. No significant difference is evident between the chlorophyll values of houses 1, 2, and 3 on either the green- or dry-weight basis. From this it may be concluded that the ultra violet of sunlight has little effect on chlorophyll. Houses 4 and 5 tend to be slightly lower than houses 1, 2, and 3, indicating that cutting out the blue rays slightly reduces chlorophyll production. Odds are 37 : 1 and 48 : 1 that chlorophyll is lower in house 5 than in house 1 on the green- and dry-weight basis, respectively.

The  $a/b$  ratio is lower in houses 4 and 5. The odds that house 5 is lower than house 1 are 78 : 1.

The total carotinoids show no very significant differences. The  $c/x$  ratio seems to be slightly higher in house 1 than in the other houses, but this cannot be ascribed to light quality, since house 3, where all the ultra violet is cut out is slightly lower than house 2.

The comparative values for the brown pigment show that it is lower in the houses lacking in blue light.

In the second series a blue glass was introduced. No shading cloth was used, and therefore the intensity differences are much greater in this series than in the previous one. This should be remembered when interpreting the data.

The conditions were as follows:

H<sub>2</sub>. Intensity about 45 percent of outside.

H<sub>3</sub>. Intensity about 45 percent of outside.

H<sub>5</sub>. Intensity about 27 percent of outside.

H<sub>6</sub>. A blue glass cutting out all wave lengths longer than 585 m $\mu$ . Intensity about 10 percent of outside.

The results of this series are tabulated in table 7. There is not much difference between house 2 and 3 in total chlorophyll or the  $a/b$  ratio. With the exception of one set of analyses, chlorophyll is higher in house 5 than in house 1. House 6 is very much higher in chlorophyll than either house 2 or

TABLE 7. *Analyses in Colored Glass Experiment where Total Intensity Varies\**

Plant	Substance Determined	Method Used	Total Pigment in percent of Fresh Weight				Total Pigment in percent of Dry Weight				Ratio, Either a/b or c/x			
			H <sub>2</sub>	H <sub>3</sub>	H <sub>4</sub>	H <sub>5</sub>	H <sub>2</sub>	H <sub>3</sub>	H <sub>4</sub>	H <sub>5</sub>	H <sub>2</sub>	H <sub>3</sub>	H <sub>4</sub>	H <sub>5</sub>
Tomato.....	Chlorophyll	Willstätter's	0.141	0.141	0.214	1.34	1.19	1.82	1.89	3.0	3.0	2.9	3.3	
".....	"	Total	0.192	0.205	0.295	1.83	1.62	2.56	2.61					
".....	"	Willstätter's	0.137	0.170	0.237	1.18	1.32	1.94	1.80	2.9	3.2	2.9	3.4	
".....	"	Total	0.183	0.241	0.324	1.58	1.82	2.74	2.46					
Soy bean.....	"	New	0.205	—	0.306	1.54	—	1.54	—	2.8	—	2.8	2.8	
".....	"	Willstätter's	0.268	0.210	—	1.07	—	0.81	—	2.8	—	2.4		
Tomato.....	Carotinoids	Willstätter's	.0154 (.0190)**	.0158 (.0190)	.0259 (.0306)	0.147 (0.181)	0.142 (0.169)	0.198 (0.238)	0.229 (0.271)	0.38 (0.31)	0.42 (0.30)	0.35 (0.26)	0.39 (0.33)	
".....	"	"	.0157	.0204	.0339	0.136	0.166	0.232	0.257	0.40	0.40	0.39	0.37	
Soy bean.....	"	"	.0334 (.0412)	.0269 (.0323)	—	0.133 (0.164)	—	0.104 (0.125)	—	0.41 (0.33)	—	0.29	0.27	
Tomato.....	Brown pigment †		1.21	0.43	0.65									
Soy bean.....	"		1.00	0.80	—									

\* H<sub>2</sub>, 45 percent of outdoor intensity.H<sub>3</sub>, 45 " " " "H<sub>4</sub>, 27 " " " "H<sub>5</sub>, 10 " " " "

\*\* Values in parenthesis are determined with spectrophotometer.

† Relative values.

house 3. Odds are 360 : 1 on the green weight basis and 832 : 1 on the dry weight basis. In house 6 the plants made very little growth. The high amount of chlorophyll is probably due to production of tissue not keeping pace with chlorophyll production. This cannot be ascribed to blue light alone, since a large intensity difference is involved. It will be shown later that reduction in intensity leads to a marked increase in chlorophyll percentage. In accord with what was noted in the previous series, the  $a/b$  ratio averages slightly lower in house 5 than in houses 2 and 3. House 6 runs slightly higher. Due to the slow growth of plants in this house, more analyses could not be made.

The total carotinoids show small changes that in general parallel those of total chlorophyll. There is an especially large increase in house 6. No significant change is shown in the  $c/x$  ratio.

The brown pigment is lower in houses 5 and 6 than in houses 2 or 3. In house 6, however, it is higher than in house 5 in spite of the lower intensity in house 6. This is in accord with the conclusion that the production of this pigment is favored by blue light.

In order to check the results in house 5 in relation to the reduction of the  $a/b$  ratio under these conditions a third series was run. Plants grown under ordinary greenhouse conditions were placed in house 5 and, after several weeks, analyses were made on them, and upon the controls left under greenhouse glass. The results are summarized in table 8. They show an undoubted decrease of the  $a/b$  ratio in house 5. The odds are over 5,000 : 1 in favor of this. Total chlorophyll expressed on the fresh weight basis shows a slight decrease in house 5. This decrease falls almost entirely on chlorophyll  $a$ . The odds that this decrease is significant are 200 : 1. On the dry weight basis, total chlorophyll remains practically unchanged. Whether the red light affects the  $a/b$  ratio by increasing the rate of decomposition of  $a$ , by causing a transformation of  $a$  into  $b$ , or by slowing up the production of  $a$ , cannot be decided by the data at hand.

Recently Sayre (1928) has shown that chlorophyll development ceases when light shorter than 680  $m\mu$  is cut out.

#### Experiment 6. Effect of Reducing the Light Intensity

In this experiment the purpose was to see what effect a reduction of intensity would have on the pigments. Plants were grown under a framework covered with black cheese-cloth. This reduced the intensity to about 12 percent of that outside.

The results of the analyses are shown in table 9. Both total chlorophyll and total carotinoids are much higher with reduced intensity on both the fresh and dry weight basis. There is no significant change in either the  $a/b$  or  $c/x$  ratios. The brown pigment is much reduced by shading.

Work of Willstätter (1912) shows that shade leaves are higher in total chlorophyll than sun leaves. This has also been shown by Lubimenko



TABLE 8. *Analyses of Plants Placed Under Glass Transmitting no Blue, Compared with Controls Left Under Window Glass*

Plant	Days Under Exp.	Method Used	Chlorophyll a in percent of Fresh Weight		Chlorophyll b in percent of Fresh Weight		Total Chlorophyll in percent of Fresh Weight		Total Chlorophyll in percent of Dry Weight		a/b Ratio	
			Control	Light Longer than 526 m $\mu$	Control	Light Longer than 526 m $\mu$	Control	Light Longer than 526 m $\mu$	Control	Light Longer than 526 m $\mu$	Control	Light Longer than 526 m $\mu$
Sunflower.....	14	New	0.114	0.110	.034	.037	0.148	0.147	0.91	1.29	3.4	3.0
".....	20	"	0.143	0.141	.033	.037	0.176	0.178	1.28	1.60	4.4	3.9
".....	21	"	0.150	0.133	.043	.038	0.193	0.171	1.30	1.54	3.6	3.6
".....	23	"	0.134	0.127	.043	.041	0.177	0.168	1.29	1.26	3.2	3.2
".....	21	Willstätter's	0.140	0.122	.043	.042	0.181	0.164	1.32	1.23	3.5	3.0
".....	21	Willstätter's	0.084	0.116	.029	.042	0.113	0.158	0.76	1.19	2.9	2.8
".....	21	Willstätter's	0.081	0.063	.028	.025	0.109	0.088	0.73	0.66	2.7	2.6
".....	21	Willstätter's	0.068	0.068	.028	.026	0.223	0.094	0.90	0.71	3.1	2.7
Soy bean.....	29	"	0.168	0.126	.055	.051	0.220	0.177	0.90	0.84	2.9	2.5
".....	29	"	0.163	0.126	.057	.054	0.319	0.180	0.89	0.85	3.2	2.9
Soy bean.....	29	New	0.242	0.201	.077	.067	0.324	0.268	1.28	1.27	3.2	3.0
Soy bean.....	29	New	0.246	0.194	.078	.068	0.324	0.262	1.30	1.24	3.2	2.9

(1907). Willstätter finds with certain plants a reduction of the  $a/b$  ratio. With all plants studied he noted a reduction of the  $c/x$  ratio. Absence of a shift in these ratios in this experiment is hard to reconcile with his results. It may be that factors other than intensity are involved in the changes observed under natural conditions.

TABLE 9. *Analyses of Plants Subjected to Reduced Intensity Compared with Plants Left in Open Sunlight\**

Plant	Substance Determined	Method Used	Total Pigment in percent of Fresh Weight		Total Pigment in percent of Dry Weight		Ratio, Either $a/b$ or $c/x$	
			Outside	Shade	Outside	Shade	Outside	Shade
Sunflower	Chlorophyll	Willstätter's	.062	0.104	0.38	0.92	2.5	2.6
Tomato...	"	"	.086	0.150	0.80	1.28	2.8	2.6
Sunflower	Carotinoids	New	.120	0.150	0.76	1.25	3.4	3.3
"		Willstätter's	.0092 (.0090)	.0121 (.0148)	0.057 (0.055)	0.108 (0.132)	0.42 (0.32)	0.42 (0.29)
Tomato...	"	"	.0112 (.0112)	.0204 (.0248)	0.104 (0.104)	0.174 (0.212)	0.42 (0.29)	0.45 (0.31)
Tomato...	Brown pigment †		2.10	1.00				
Sunflower	"		2.10	1.00				

\* Values in parenthesis determined with spectrophotometer.

† Relative values.

### Experiment 7. Effect of Mineral Nutrients

These experiments were conducted with soy beans grown in quartz sand and watered with nutrient solutions and distilled water. Oats were also grown in minus potassium and control cultures, since Wlodek claims to have found evidence of a shift of the  $a/b$  ratio with this treatment, using oats as the experimental plant.

The nutrients<sup>3</sup> were prepared in the following manner:

#### STOCK SOLUTIONS

1.  $MgSO_4 \cdot 7H_2O$ ..... 2 percent
2.  $KNO_3$ ..... 2 percent
3.  $Ca(NO_3)_2 \cdot 4H_2O$ ..... 4 percent
4.  $K_2HPO_4$ ..... 2 percent
5.  $KCl$ ..... 2 percent
6.  $CaCl_2$ ..... 2 percent
7.  $NaNO_3$ ..... 2 percent
8.  $Na_2HPO_4$ ..... 2 percent

For complete nutrients use equal parts of 1, 2, 3 and 4.

For minus phosphorus use equal parts of 1, 2, 3 and 5.

For minus nitrogen use equal parts of 1, 4, 5 and 6.

For minus potassium use equal parts of 1, 3, 7 and 8.

To make the nutrients, 10-cc. portions of the solutions were mixed as

<sup>3</sup> I am indebted to Mr. J. T. Sullivan of Purdue University for suggesting this combination of nutrient salts.

TABLE 10. *Analyses in Experiment with Mineral Nutrients*

Plant	Age in Days	Substance Determined	Method Used	Total Pigment in percent of Fresh Weight					Total Pigment in percent of Dry Weight					Ratio, Either a/b or c/x				
				Soil	Com- plete	- N	- P	- K	Soil	Com- plete	- N	- P	- K	Soil	Com- plete	- N	- P	- K
boy bean *	39	Chlorophyll	Willstätter's	0.186	0.115	0.150	0.139	0.153	1.05	0.89	0.95	0.83	0.92	2.8	2.1	2.2	3.0	2.2
" "	42	"	"	0.209	0.160	0.183	0.166	0.146	1.21	1.15	1.08	1.12	1.12	2.4	2.6	2.2	2.7	2.4
lats *	46	"	"		0.147			0.170	1.83	1.43			1.40		3.8			3.9
boy bean †	36	"	New	0.321	0.177	0.141	0.211		1.83	1.06	0.65	1.02		3.3	3.1	3.2	3.2	
" "	41	"	"	0.229	0.166		0.214		1.15	0.81		0.95		4.2	4.4		3.9	
" "	36	"	Willstätter's	0.194	0.176	0.107	0.181		1.10	1.05	0.49	0.87		3.4	3.2	3.2	2.8	
" "	41	"	"	0.184	0.112	0.082	0.119		0.92	0.55	0.32	0.53		3.1	3.1	3.2	2.4	
boy bean *	42	Carotinoids	Willstätter's	0.0267	0.0187	0.0206	0.0175	0.0163	0.154	0.135	0.122	0.119	0.125	0.41	0.47	0.52	0.44	0.46
lats *	46	"	"		0.0198			0.0238		0.194			0.197		0.54			0.58
boy bean †	36	"	"	0.0275	0.0212	0.0137	0.0256		0.157	0.127	0.063	0.124		0.37	0.39	0.47	0.35	
" "	41	"	"	0.0247	0.0162	0.0099	0.0205		0.124	0.079	0.039	0.091		0.42	0.39	0.46	0.35	
boy bean †	36	Brown Pigment †		1.00	2.39	2.95	2.28											
" "	41	"		1.00	2.17	2.48	2.06											

\* Analyses on plants from seed planted December 9, 1926.

† Analyses on plants from seed planted April 21, 1927.

‡ Relative values.

indicated above and diluted with 250 cc. of distilled water. A few drops of a solution of ferric citrate was added in each case. Applications were made each week.

The results are summarized in table 10. Two lots of plants were grown, the first series being started on December 9, 1926, and the other April 21, 1927. The light intensity in these two series was extremely different and the response to reduction of the nitrate supply was not the same in both. Therefore, the results of these series will be considered separately.

In the winter series total chlorophyll expressed on the fresh weight basis is higher where an essential element is lacking. Odds for minus nitrogen are 100 : 1, for minus phosphorus 16 : 1, and for minus potassium 14 : 1. Plants grown in soil are distinctly higher in total chlorophyll than if grown in the complete nutrient solution, both on the dry and fresh weight basis. The odds are 1,660 : 1 and 140 : 1, respectively. The  $a/b$  ratio shows no changes that can be regarded as significant. Total carotinoids show changes that parallel that of total chlorophyll. Unfortunately, no comparison of the brown pigment was made in this series.

In the spring series only soil, complete, minus nitrogen and minus phosphorus cultures were grown. It is evident that here the action of lack of nitrates is decidedly different from that in winter. There is a marked decrease in total chlorophyll on both the fresh and dry weight basis. The odds are 450 : 1 and 1,600 : 1, respectively. The soil and minus phosphorus cultures show essentially the same effects on total chlorophyll as did the winter series. It is very interesting that total carotinoids as well as total chlorophyll decrease in the minus nitrogen cultures. In the soil and minus phosphorus cultures they are higher than in the complete solution on the fresh weight basis. The brown pigment shows large changes, which are inversely correlated with total chlorophyll, soil being lowest and minus nitrogen highest.

Considering the  $a/b$  and  $c/x$  ratios for both series together, there is no evidence of significant differences except for the slightly high  $c/x$  ratios with minus nitrogen and the somewhat low values with minus phosphorus.

The difference of the effect of nitrates in the two series is probably to be explained on the basis of an increased light intensity and duration in the spring, leading to the production of abundant carbohydrates and consequently making nitrogen a limiting factor. In the winter with lower carbohydrates, the nitrogen in the seed sufficed to provide for the needs of the plant. Another possibility to consider, is the probable action of light to decompose chlorophyll in the leaf. This action would be greater in the spring and tend to reduce the chlorophyll percentage. It would be expected that the amount of nitrogen supplied by the seed might be sufficient to produce chlorophyll rapidly enough to care for its decomposition in the winter with low light intensity, but not in the spring with high light intensity.

Deuber (1928) has also noted that lack of nitrogen may increase chlorophyll in soy beans under certain conditions.

The action of lack of phosphorus and potassium may be due to the decreased growth of the plant, chlorophyll production not being similarly affected. This may also partially explain the action of soil, since the plants in the soil cultures grew slower than with the complete nutrient solutions. It may be possible that certain accessory nutrients were present in the soil and not in the culture solutions.

### Experiment 8. Effect of Continued Darkness

When soy beans or tomatoes are placed in the dark room there is a marked reduction in total chlorophyll in a few days. The results of experiments of this nature are summarized in table 11. The reduction in total chlorophyll is evident without recourse to analysis, but its magnitude is shown by the data. Carotinoids do not show a parallel decrease. Aside from the second analysis on soy beans they remain almost constant.

The most interesting result of these experiments is the large increase in the  $c/x$  ratio in the plants kept in darkness. This appears to be due to a decrease in xanthophyll and an increase in carotin. It looks as if xanthophyll had been changed to carotin, but the data do not exclude other possibilities. The brown pigment shows a marked increase with continued darkness.

Yellow *Coleus* was investigated as a plant naturally low in chlorophyll. It does not show the same changes as the other plants. Neither total chlorophyll nor total carotinoids show changes. Both are quite low in this plant. The brown pigment does not change much. The dilute acetone extract of this plant is in general darker than usually found. It seems also to differ qualitatively from that of tomato and soy bean. The  $c/x$  ratio is higher than commonly found. This rather high ratio in the yellow *Coleus* has been also noted by Schertz (1923).

### Experiment 9. Effect of Tomato Mosaic

A few chlorophyll analyses of mosaic tomato and tobacco plants have been published by Elmer (1925). His analyses show a great increase in the  $c/x$  ratio, carotin almost doubling in amount. It was chiefly to check this that a few analyses were made on healthy and mosaic tomatoes. No such marked change in the  $c/x$  ratio as that reported by Elmer was found. Chlorophyll seems to break down faster than the carotinoids under the action of the disease. The brown pigment shows a large increase with mosaic.

### DISCUSSION

Since many points have been treated in detail under the separate experiments, it is necessary here to indicate only the more important results and deal with conclusions that can be drawn from the data as a whole.

One of the main objects of the work was to determine whether the  $a/b$

TABLE II. *Analyses on Plants Placed in Darkness Compared with Plants Left in Light*

Plant	Days Under Exp.	Total Chloro. in percent of Fresh Wt.		Total Chloro. in percent of Dry Wt.		Rel. Am't. of Brown Pigment		Carotin in percent of Fresh Wt.		Xanthophyll in percent of Fresh Wt.		Total Carot. in percent of Fresh Wt.		Total Carot. in percent of Dry Wt.		c/k Ratio	
		Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Soy bean.....	8	0.264	0.062	1.16	0.33	1.00	2.91	.0049 (.0040)*	.0096 (.0081)	.0164 (.0149)	.0123 (.0081)	.0213 (.0189)	.0219 (.0162)	0.113 (0.100)	0.096 (0.071)	0.32 (0.29)	0.82 (1.07)
" ".....	8	0.368	0.098	1.65	0.59	1.00	3.39	.0091 (.0081)	.0068 (.0061)	.0297 (.0267)	.0143 (.0137)	.0388 (.0348)	.0211 (.0200)	0.174 (0.156)	0.128 (0.121)	0.34 (0.32)	0.51 (0.49)
Tomato.....	4	0.185	0.146	2.25	1.64	1.00	1.56	.0042	.0049	.0139	.0121	.0181	.0170	0.220	0.192	0.34	0.46
Yellow Coleus.	8	0.023	0.022	0.25	0.31	1.00	0.81	.0021	.0021	.0034	.0040	.0055	.0061	0.059	0.085	0.65	0.56

\* Values in parenthesis determined with spectrophotometer.

and  $c/x$  ratios could be changed. It is shown that storing at a temperature below freezing will cause a lowering in the  $a/b$  ratio. Cutting out the blue rays from sunlight will also produce a lowering of this ratio. Both the  $a/b$  and  $c/x$  ratios decrease when tomatoes are placed under continuous illumination. An elevation of the  $c/x$  ratio occurs when plants are placed in total darkness.

The fact that the ratios of the pigments to each other can be changed eliminates the likelihood of their existence in the leaf combined with each other in definite proportions. It is reasonable to assume that the difficulty of shifting the ratios has some explanation, as does also the small changes produced by certain conditions.

It is interesting to consider a possibility that arises in view of what has been found in this work. Suppose that chlorophyll  $a$  and chlorophyll  $b$  exist in the leaf in equilibrium with each other as might be indicated by the following:



It would be reasonable to expect that the reaction to the right would evolve heat. According to the theorem of Le Chatelier, lowering the temperature should shift the equilibrium toward the right, increasing chlorophyll  $b$ . In agreement with this,  $b$  increases and  $a$  decreases when leaves are stored at a temperature below freezing. It is very unfortunate that there were no other experiments on the effect of temperature. Experiments 1 and 3 were at 25° C, while experiment 2 was at 20° C. The  $a/b$  ratios were lower in experiment 2. Since these analyses were separated over a period of time, a constant error may be involved. However, the indications are sufficiently favorable to warrant further work on the effect of temperature.

Spectrograms of chlorophyll  $a$  and chlorophyll  $b$ , as given by Willstätter, show that chlorophyll  $a$  absorbs more red light than chlorophyll  $b$ , and chlorophyll  $b$  somewhat more blue than chlorophyll  $a$ . It would be expected, therefore, that a preponderance of red light would increase the activity of chlorophyll  $a$  more than  $b$  and shift the above postulated equilibrium to the right. Similarly a preponderance of blue light should increase  $a$ . This is in agreement with the data.

The ratio changes in the case of tomatoes can be best explained on the basis of the pigments decomposing at different rates under these conditions. The conversion of chlorophyll  $a$  to chlorophyll  $b$  and of carotin to xanthophyll by the process of oxidation under conditions of rapid photosynthesis is not to be excluded, since the relative amounts of the more highly oxidized pigments increase. Willstätter (1918) has observed that the  $c/x$  ratio is lowered when detached leaves are placed under continuous artificial illumination and high carbon dioxide concentration. These conditions are very

favorable to oxidation. He kept leaves at the same temperature in darkness and found no shift in the  $c/x$  ratio. Illuminated leaves that had been treated with ether to stop photosynthesis showed a normal  $c/x$  ratio. It is probable that the change observed is due to conversion of carotin to a product that at least behaves similarly to xanthophyll in the analysis, Willstätter regards this change as similar to that which frequently occurs during autumnal coloration.

It was found that long continued darkness results in a decrease in chlorophyll without a corresponding decrease in carotinoids. It seems that an increased rate of decomposition, favored by the conditions that darkness induces in the leaf, occurs, rather than the decrease being due solely to the stopping of chlorophyll production with decomposition going on at a normal rate. However, the most interesting result of darkness is an increase in the  $c/x$  ratio, a result which might be expected when the evolution of oxygen is checked due to photosynthesis ceasing.

It is to be noted that the shifts in the ratios observed so far result from very abnormal conditions. This must be especially borne in mind in attempting to connect these changes in any way with the mechanism of carbon assimilation. One of the strongest arguments against the inter-conversion of the pigments during photosynthesis is the result of Willstätter showing no significant diurnal fluctuations. It may be, however, that the present methods are not accurate enough to detect small changes during the course of a day.

It is interesting to note that a decrease in chlorophyll is accompanied in most cases by a decrease in carotinoids. Chlorophyll and the carotinoids also tend to increase simultaneously. The chief exception to this is the effect of continued darkness. Of especial interest is the decrease in carotinoids that accompanies a decrease in chlorophyll, due to the effect of insufficient nitrates. It is probable from this that the effect of nitrates on the amount of chlorophyll in plants is to be explained on some other basis than the fact that nitrogen enters into the chemical composition of chlorophyll. Its effect must be on some step common to the development of the carotinoids as well.

It is very difficult to draw conclusions from the values for total chlorophyll, since undoubtedly two processes which cannot be separated are involved. One is the formation of chlorophyll, the other its decomposition. Conditions that change the amount of chlorophyll may affect one or both of these processes. This complication is not confined to chlorophyll, but is involved in any attempt to interpret analyses made on plants. It would be an important step in the field of plant physiology if ways could be devised of surmounting this difficulty.

A very interesting point brought out by the data is the marked correlation of the brown pigment of the dilute acetone extract with low chlorophyll values, especially where it is likely that these low chlorophyll values



are brought about by increased decomposition. Until the effect of darkness was studied, it was thought high brown pigment values were associated with conditions favoring high carbohydrates. Darkness should result in a depletion of carbohydrates, but nevertheless, the brown pigment increases, accompanied by a decrease in chlorophyll. It is likely that the correlation of low chlorophyll and high brown pigment is due to the action of a common cause, such as a derangement of some metabolic process in the leaf, rather than the brown pigment being a chlorophyll decomposition product. A decision may be possible if the chemical properties of the brown pigment can be worked out. An attempt in this direction is being made.

### SUMMARY

1. Willstätter's fractionation method for chlorophyll *a* and *b* gives total chlorophyll values that are lower than those obtained by his method for total chlorophyll. A new method is described for fractionating chlorophyll *a* and *b* which gives higher values than those obtained by Willstätter's fractionation method. The total chlorophyll values obtained by this new method approach closely the values obtained by Willstätter's total chlorophyll method.

2. Substitute standards made from inorganic salts are described for chlorophyll determinations and new values are suggested for the potassium dichromate solution described by Willstätter as a standard for carotinoid determinations.

3. A new method is given for preparing carotin from carrots. The specific transmissive index of carotin at the mercury line 435.8 was found to be 2.11.

4. A method for determining the relative amounts of a brown pigment that may be extracted from leaves with dilute acetone is given.

5. Total chlorophyll does not change significantly during storage of tissue in the frozen condition, but the *a/b* ratio decreases.

6. Increasing the concentration of carbon dioxide in the air results in a decrease in total chlorophyll and carotinoids on the dry weight basis, but little change on the fresh weight basis. It does not change the *a/b* or *c/x* ratios.

7. Increasing the duration of illumination results in a decrease in total chlorophyll and total carotinoids, especially on the dry weight basis. There is no significant shift in the *a/b* or *c/x* ratios.

8. When both extra carbon dioxide and increased day length are applied simultaneously, the decrease in total chlorophyll and total carotinoids is greater. The *a/b* and *c/x* ratios remain unchanged.

9. Very young plants, such as seedling soy beans, are exceptions to the above, extra carbon dioxide bringing about an increase in chlorophyll.

10. In tomatoes subjected to continuous artificial illumination there is a

marked reduction in chlorophyll and carotinoids in a few days. The  $a/b$  and  $c/x$  ratios are both significantly lowered. The brown pigment increases.

11. Cutting of the ultra violet from sunlight has no significant effect on chlorophyll or carotinoids.

12. Elimination of blue light results in a slight but significant decrease in chlorophyll and carotinoids, provided the intensity is kept the same as the control. The  $a/b$  ratio is lowered by cutting out blue light.

13. Cutting out red light results in an increase in both chlorophyll and carotinoids, but this is due partially to intensity reduction. The  $a/b$  ratio is higher under the blue light, but the number of analyses is insufficient to be certain of this increase. Brown pigment production is favored by blue light.

14. Reducing the light intensity to 12 percent of normal sunlight results in an increase in chlorophyll and carotinoids. The  $a/b$  or  $c/x$  ratios show no significant change. Brown pigment is reduced.

15. Removing the nitrate supply leads to increased chlorophyll and carotinoid percentages on the green weight basis, under the light conditions prevailing in the greenhouse during the winter. The opposite is true in the spring, with higher light intensity. Under these latter conditions, brown pigment increases.

16. Removing the potassium or phosphorus supply leads to an increase in chlorophyll on the green weight basis, but little change on the dry weight basis.

17. Chlorophyll and carotinoids are markedly higher in soil than in any of the nutrient solutions used. Brown pigment is low in plants grown in soil.

18. Lack of nitrates, phosphorus, or potassium does not significantly affect either the  $a/b$  or  $c/x$  ratios.

19. Placing plants in the dark results in a large decrease in chlorophyll, unaccompanied by a corresponding decrease in carotinoids. The  $c/x$  ratio shows a marked increase. Brown pigment increases.

20. Tomato mosaic results in a decrease in chlorophyll, which may be accomplished by a decrease in carotinoids. Brown pigment increases. The  $a/b$  and  $c/x$  ratios show no large changes.

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## RELATION OF COMPOSITION OF SEED AND THE EFFECTS OF LIGHT TO GROWTH OF SEEDLINGS

MARY E. REID

It is possible to study the effect of light on the assimilation of inorganic nitrogen, the synthesis into proteins of the products formed therefrom, and the utilization of these products in growth independently of carbohydrate synthesis. This can be done by growing plants in the light in an atmosphere lacking carbon dioxid. A plant may have a supply of reserve carbohydrates or fats sufficient to last for a considerable time. Such a reserve may be adequate to afford energy for respiration and building material for protoplasm and cell walls without having the complicating influence of carbohydrate synthesis. Under such circumstances is growth in the light the same as in darkness or is there a distinct consequence of light on growth and development aside from its rôle in synthesizing carbon compounds? Since there is now much evidence that varying proportions of carbohydrates and nitrogen have remarkable influences on metabolism and growth, may it not be that by controlling the process of carbohydrate synthesis, extreme variations in the relative proportions of carbohydrates to nitrogen may be obtained with the probability of resulting effects upon the type of development?

Godlewski (2) investigated the process of germination in the light without the presence of carbon dioxid and found that it went on much the same as with seedlings grown in the normal atmosphere. There was no stretching out of the stem as occurs with plants grown in darkness. The chloroplasts were completely formed and of normal color. He concluded that the form changes of etiolated plants are not to be sought in the interruption of carbohydrate synthesis.

Corenwinder (1) enclosed a leafy branch of a tree in a glass cylinder filled with air lacking carbon dioxid. The leaves of the tree continued to grow and eventually became larger than those kept in the normal atmosphere. He attributed the difference in size of the two sets of leaves to the more favorable temperature for growth which he thought existed within the chamber. It was concluded that the leaf assimilated the carbon dioxid given off by its own tissues or which was probably brought to it from other parts of the plant. Undoubtedly growth occurred at the expense of

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carbohydrate compounds stored locally within the stem and possibly also some was conducted in from the adjoining portion of the branch. From a consideration of the results of the present experiments it seems possible that the limitation of carbohydrate synthesis may itself have had a favorable effect on the growth of the leaves enclosed within the chamber.

Godlewski (3) claimed that the daily growth period is, as Sachs indicated, brought about by the retarding effect of light on growth. That both internodes and leaves show this retarding effect is indicated by Prantl's (9) and Vines' (12) measurements. Godlewski thought the retarding of growth by light is a consequence of its effect on the protoplasm but he considered that the decreased growth could not be entirely explained by a lessened motility of the protoplasmic molecules. He held also that the extensibility of the cell walls is decreased by light.

Kraus (5) attributed the limited thickening of tissues in darkness to lack of carbohydrate synthesis. He claimed that light influences the thickening of the tissues because it makes possible the formation of carbohydrates. Godlewski, on the contrary, maintained that thickening of the tissues goes on if the plants are grown in a carbon-dioxid-free atmosphere. He held that the effect of light on the solidification and thickening of the plant membranes has nothing to do with carbohydrate synthesis.

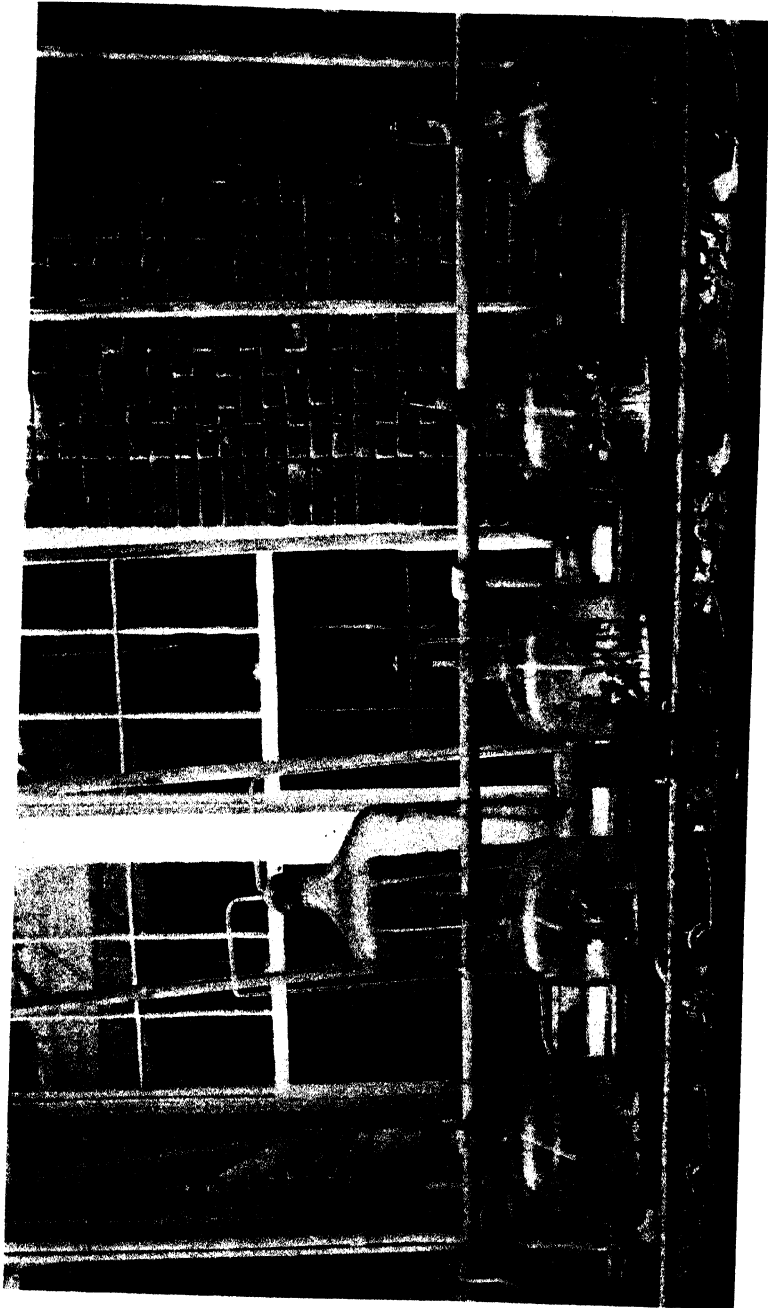
The literature dealing with the relation of varying proportions of carbohydrates and nitrogen to growth of shoots and roots has been reviewed in previous publications (Reid, 10, 11).

Most of the investigators in this field of research, particularly Godlewski (4) and Pfeffer (8), have been chiefly interested in the chemical differences of plants grown in darkness and in light with and without carbon dioxid and they have not described the type of growth with any detail. Very little attention has been given to the relative weights of different organs of the plant, the number and kind of roots produced, the color and size of cotyledons and leaves, and differences in anatomical structure of plants grown in darkness and in light in atmospheres having and lacking carbon dioxid.

#### EXPERIMENTAL METHODS

Seedlings of Illinois high- and low-protein corn, cow pea, soy bean, muskmelon, and sunflower were used. The cultures were prepared according to methods described in a previous publication (Reid, 11). When the seedlings were beginning to emerge from the sand, the cultures were placed in the apparatus devised for (a) providing an atmosphere containing a relatively high concentration of carbon dioxid, and (b) an atmosphere lacking carbon dioxid.

This apparatus consisted of a series of 12 tubulated bell jars, six of which were used as culture chambers having the atmosphere containing carbon dioxid and the other six were used as chambers in which the carbon dioxid of the incoming air was completely removed. Text figure 1 shows one of the



TEXT FIG. 1. Apparatus for controlling concentration of carbon dioxide.

two series. The lower outlet tube of each jar was connected with a gas pipe having six outlets, one leading to each jar. The gas pipe was connected to the vacuum system of the building and by means of a screw the amount of vacuum could be regulated. The tubulated openings at the tops of the jars were individually connected with another gas pipe on the opposite side of the jars. The air leading into this gas pipe was, in one series, conducted through a soda lime tube and then through baryta water before entering the gas pipe leading to the culture chambers. In the other series the air was conducted through a strip of glass tubing about one inch in diameter and thirty inches long. Extending into this open tube was a smaller tube leading from a carbon dioxide tank.

The pressure from the tank was kept uniform by means of a pressure gauge and the gas was allowed to bubble out at the rate of 20 bubbles per minute. The rate of flow was checked at frequent intervals during the day by holding the end of the small tube under water and counting the number of bubbles per minute. It was found that with a vacuum which had a force sufficient to lift a column of water 6 cm. and by allowing carbon dioxide to enter the current of ingoing air in the large tube at the rate of 20 bubbles per minute, the air in the culture chambers was kept at a concentration of approximately 0.4 percent carbon dioxide (by volume).

The jars were sealed with DeKhotinsky cement to glass plates 12 inches square. It was necessary to maintain an air-tight seal in order to insure an equal pull of air through all of the jars. At frequent intervals a water manometer was inserted into the system at the inlet and outlet of each jar to make certain that the pull was uniform in all of the chambers.

Fresh applications of nutrient solutions were made through a glass tube inserted through the rubber stopper at the top of each jar. The tube extended to within an inch of the level of the sand and was kept closed by a screw pinch cock at the exposed end except when nutrient solution or distilled water was to be applied.

The investigations were conducted chiefly during May and June, when light supposedly would not be a limiting factor. Experiments have also been made at other times during the year but the results, although interesting, are not sufficiently conclusive to report at this time.

The seedlings were allowed to develop until the plants grown without extra carbon began to show evidences of loss of turgor, etc., resulting undoubtedly from insufficient carbohydrates. The plants of any particular kind, receiving and not receiving carbon dioxide, were always harvested at the same time. Due to differences in the relative amounts of reserve carbohydrates and to variations in the rate of growth of different types, the length of the period of growth varied with the kind of plant. Illinois high-protein corn, for example, grown without extra nitrogen or extra carbon had a growing period of only 9 days but sunflower seedlings under the same conditions grew for 14 days. When given extra nitrogen, the high-protein

corn grew for ten days, whereas soy bean seedlings grew for 17 days. Throughout the developmental period light conditions were favorable for rapid growth and synthesis of carbohydrates.

Microchemical tests on sections of tissues of seedlings grown under the different external conditions were used for the detection of starch, free-reducing substances, cellulose, and lignin. The following methods were used:

*Starch.* Dilute solution of IKI. Starch grains stained deep blue.

*Free-reducing substances.* Flückiger reaction: a few granules of copper tartrate were placed on a slide and dissolved in a 20-percent solution of NaOH. After standing for 10 minutes at 45° C. the tissues were examined for copper oxid crystals.

*Cellulose.* Sections of tissue were placed on a slide in a drop of I<sub>2</sub>KI solution and excess of the reagent removed with filter paper. The location of the blue color in the sections was observed. A drop of 75-percent H<sub>2</sub>SO<sub>4</sub> was then added from the side of the cover glass. Cellulose membranes will swell and become blue in the reagent.

*Lignin.* Phloroglucin-HCl reaction. Sections of tissue were placed on a slide in alcoholic phloroglucin (0.1 gm. phloroglucin, 10 cc. alcohol); the sections were covered and left until the solution evaporated. Then a drop of 25-percent HCl was added from the edge of the cover glass. A red-violet color in the cell walls was taken as an indication of the presence of lignin.

Godlewski (4) and Vines (12) have both maintained that leaves could be developed in light under conditions which inhibited any assimilation of carbohydrates. In the experiments herein described, the air entering six of the chambers was free from carbon dioxide but the air in the chambers themselves contained small amounts which must have been produced in respiration. The concentration of carbon dioxide in the tissues of the leaves was probably considerably higher than that in the air surrounding the plants in the chambers. Although at the time the plants were harvested starch was not found in the leaves except in the guard cells and bundle sheaths, it is not supposed that carbohydrate synthesis was completely inhibited. There may have been considerable utilization of the carbon dioxide followed by growth with accompanying respiration. Toward the end of the growing period the amount of carbon dioxide in the air of the culture chambers must have been very small. Determinations of the carbon dioxide were not made during the later phases of growth of the seedlings, consequently this question cannot be answered.

## RESULTS OF EXPERIMENTS

The results of these experiments show the effect of differences in chemical composition of the seed upon the growth of the seedling, particularly in relation to the availability or non-availability of additional carbon and nitrogen. The growth responses of each type of seedling will be described.



TABLE I. Seedlings from High and Low Protein Seeds Grown Without Nitrates and With and Without Carbon Dioxid, June 1-14, 1925

Plant	Variety	Length of Growth Period Days	Number of Plants	Total Nitrogen in Seeds %	Weight of Seeds	Green Weights per Plant				
						Roots gms.	Stems gms.	Leaves + Cots. gms.	Shoots (Stems + Leaves) gms.	Entire Plant gms.
Carbon Dioxid Removed from Incoming Air										
*Corn.....	Illinois high-protein	9	10	2.76	2.73	1.136	+ leaf sheaths	0.791	1.320	2.46
Corn.....	Illinois low-protein	10	10	1.09	3.91	1.082	+ leaf sheaths	0.516	1.010	2.09
Cow peas.....	New Era	12	8	4.40	1.04	0.220	0.494	0.276	0.620	0.840
Soy bean.....	Peking	13	14	3.40	0.94	0.183	0.244	+ cots	0.566	0.749
Muskmelon.....	Red Rocky Ford	12	24	5.62	0.32	0.089	0.058	0.321 cots only	0.203	0.292
Sunflower.....	Mammoth Russian	14	12	4.56	0.57	0.157	0.393	0.145 cots only	0.678	0.835
Incoming Air Containing 0.3-0.4% Carbon Dioxid										
Corn.....	Illinois high-protein	9	10	2.76	2.73	1.155	0.792	0.902	1.694	2.85
Corn.....	Illinois low-protein	10	10	1.09	3.91	0.991	0.584	0.441	1.025	2.02
Cow peas.....	New Era	12	8	4.40	1.04	0.756	0.731	cots shed	1.292	2.05
Soy bean.....	Peking	13	14	3.40	0.94	0.640	0.388	0.561	0.840	1.48
Muskmelon....	Red Rocky Ford	12	24	5.62	0.32	0.349	0.133	+ cots	0.377	0.726
Sunflower.....	Mammoth Russian	14	12	4.56	0.57	0.462	0.818	0.244 cots only	1.228	1.69

TABLE 2. Seedlings from High and Low Protein Seeds Grown With Nitrates and With and Without Carbon Dioxid, May 12-29, 1925

Plant	Variety	Length of Growth Period, Days	Number of Plants	Total Nitrogen in Seeds %	Weight of Seeds	Green Weights per Plant				
						Roots gms.	Stems gms.	Leaves + Cots. gms.	Shoots (Stems + Leaves) gms.	Entire Plant gms.
Carbon Dioxide Removed from Incoming Air										
Corn.....	Illinois high-protein	10	10	2.76	2.73	1.112	+ leaf sheaths 0.577	0.927	1.504	2.62
Corn.....	Illinois low-protein	15	10	1.09	3.91	1.660	+ leaf sheaths 0.784	1.291	2.075	3.74
Cow peas.....	New Era	14	10	4.40	1.30	0.442	0.467	0.263	0.730	1.17
Soy bean.....	Peking	17	12	3.40	0.80	0.366	0.242	+ cots 0.387	0.629	1.00
Muskmelon....	Red Rocky Ford	16	15	5.62	0.20	0.119	0.075	+ cots 0.191	0.265	0.384
Sunflower.....	Mammoth Russian	12	14	4.56	0.66	0.179	0.278	+ cots 0.294	0.573	0.752
Incoming Air Containing 0.3-0.4% Carbon Dioxide										
Corn.....	Illinois high-protein	10	10	2.76	2.73	1.280	0.787	0.958	1.745	3.03
Corn.....	Illinois low-protein	15	10	1.09	3.91	1.726	1.036	1.376	2.412	4.14
Cow peas.....	New Era	14	10	4.40	1.30	1.569	0.950	0.760	1.710	3.28
Soy bean.....	Peking	17	12	3.40	0.80	0.587	0.514	0.577	1.092	1.68
Muskmelon....	Red Rocky Ford	16	15	5.62	0.20	0.597	0.294	+ cots 0.559	0.853	1.45
Sunflower.....	Mammoth Russian	12	14	4.56	0.66	0.441	0.702	+ cots 0.626	1.328	1.77

TABLE 3. *Growth of Seedlings from Seeds of Different Nitrogen Content With and Without Carbon Dioxid and Nitrates*

Plant	Variety	Nitrogen in Seeds %	Fresh Weight (gms.) per Plant							
			Without Carbon Dioxid Without Nitrates		Without Carbon Dioxid With Nitrates		With Carbon Dioxid Without Nitrates		With Carbon Dioxid With Nitrates	
			Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Corn.....	Illinois low-protein	1.09	1.010	1.082	2.075	1.660	1.025	0.991	2.412	1.726
Corn.....	Illinois high-protein	2.76	1.320	1.136	1.504	1.112	1.694	1.155	1.745	1.280
Soy bean.....	Peking	3.40	0.566	0.183	0.629	0.366	0.840	0.640	1.092	0.587
Cow peas.....	New Era	4.40	0.620	0.220	0.730	0.442	1.292	0.756	1.710	1.569
Sunflower.....	Mammoth Russian	4.56	0.678	0.157	0.573	0.179	1.228	0.462	1.328	0.441
Muskmelon.....	Red Rocky Ford	5.62	0.203	0.089	0.265	0.119	0.377	0.349	0.853	0.597

TABLE 4. *Percentage Gain \* in Fresh Weight of Seedlings When Nitrates or Carbon Dioxid or Both are Supplied*

Plant	Variety	Nitrogen in Seeds %	Nitrates Supplied			Carbon Dioxid Supplied			Nitrates and Carbon Dioxid Supplied		
			Roots	Stems	Leaves + Cots.	Roots	Stems	Leaves + Cots.	Roots	Stems	Leaves + Cots.
Corn.....	Illinois high-protein	2.76	- 2	+ 9	+ 17	+ 1	+ 50	+ 14	+ 13	+ 49	+ 21
Corn.....	Illinois low-protein	1.09	+ 53	+ 59	+ 150	- 9	+ 18	- 14	+ 59	+ 110	+ 168
Cow peas.....	New Era	4.40	+ 100	+ 36	- 5	+ 243	+ 112	+ 103	+ 613	+ 176	+ 175
Soy beans.....	Peking	3.40	+ 100	- 1	+ 20	+ 249	+ 59	+ 40	+ 220	+ 111	+ 80
Muskmelon.....	Red Rocky Ford	5.62	+ 33	+ 29	+ 31	+ 292	+ 129	+ 68	+ 570	+ 407	+ 119
Sunflower.....	Mammoth Russian	4.56	+ 14	- 29	+ 3	+ 194	+ 108	+ 44	+ 180	+ 78	+ 119

\* The fresh weight of seedlings produced by the reserves within the seed (without additional C or N) is taken as standard.

TABLE 5. *Green Weight of Tissue Produced per 0.1 Gram of Reserve Nitrogen*

Plant	Variety	Total Nitrogen in Seeds %	Carbon Dioxid Removed from Incoming Air									
			With Nitrates					Without Nitrates				
			Total gms.	Roots gms.	Stems gms.	Leaves + Cots. gms.	Shoots (Stems + Leaves) gms.	Total gms.	Roots gms.	Stems gms.	Leaves + Cots. gms.	Shoots (Stems + Leaves) gms.
Incoming Air Containing 0.3-0.4 % Carbon Dioxid												
Corn.....	Illinois high-protein	2.76	34.74	14.77	7.66	12.31	19.97	32.62	15.09	7.02	10.50	17.53
Corn.....	Illinois low-protein	1.09	87.67	38.97	18.40	30.30	48.70	49.11	25.40	11.60	12.11	23.71
Cow peas...	New Era	4.40	23.98	7.73	8.16	4.60	16.25	14.69	3.85	6.01	4.83	10.84
Soy bean...	Peking	3.40	45.65	16.17	10.66	17.09	29.48	32.84	8.02	10.72	14.10	24.82
Muskmelon...	Red Rocky Ford	5.62	51.51	15.98	10.00	25.53	35.53	38.98	11.88	7.77	19.33	27.10
Sunflower...	Mammoth Russian	4.56	34.98	8.34	12.95	13.69	26.64	37.73	7.19	18.15	12.38	30.54
Incoming Air Containing 0.3-0.4 % Carbon Dioxid												
Corn.....	Illinois high-protein	2.76	40.17	17.00	10.45	12.72	23.17	37.84	15.34	10.52	11.97	22.50
Corn.....	Illinois low-protein	1.09	97.13	40.51	24.32	32.30	56.62	47.32	23.26	13.71	10.35	24.06
Cow peas...	New Era	4.40	57.32	27.43	16.61	13.28	29.89	35.85	13.23	12.80	9.82	22.62
Soy bean...	Peking	3.40	74.04	25.88	22.68	25.48	48.16	56.46	28.09	10.03	18.34	28.37
Muskmelon...	Red Rocky Ford	5.62	194.46	80.18	39.46	74.82	114.28	96.83	46.55	17.78	32.50	50.28
unflower...	Mammoth Russian	4.56	82.32	20.53	32.66	29.13	61.79	78.03	21.34	37.77	18.92	56.69

Photographs showing the appearance of high- and low-protein corn, muskmelon, and cow pea seedlings under each of the four sets of environmental conditions were published previously (Reid, 10).

The quantitative data are given in tables 1 to 5. Table 3 summarizes the results given in tables 1 and 2. The data presented in tables 4 and 5 are based on calculations made from tables 1 and 2. Table 4 shows the percentage gain in fresh weight of seedlings when nitrates or carbon dioxide or both are supplied. The fresh weight of seedlings produced by the reserves within the seed, without additional carbon or nitrogen, is taken as the standard.

### Illinois Low-protein Corn

These seedlings were grown from seeds containing only 1.09 percent nitrogen but a large store of carbohydrates. The amount of reserve carbohydrates in proportion to the reserve nitrogen was very much higher than in any of the other types of seeds used in these experiments. The seedlings were greatly benefited by extra nitrogen in the form of nitrates, but were not benefited (in fact showed injury) by the use of carbon dioxide unless extra nitrogen was also given. In tables 1 and 2 it is shown that plants supplied with neither carbon dioxide nor nitrates weighed 2.09 grams each; those furnished carbon dioxide but no nitrates weighed 2.02 grams each; whereas others given nitrates but no carbon dioxide weighed 3.74 grams. Seedlings supplied with both carbon dioxide and nitrates weighed 4.14 grams each. The differences in green weight of the unnitrated plants receiving and not receiving carbon dioxide are not significant although there were definite differences in appearance, especially in size of the tops. Leaves of the unnitrated seedlings grown under conditions which restricted carbohydrate synthesis were longer and more green, and there tended to be more visible leaves produced than by similar seedlings which were permitted to synthesize carbohydrates. Leaf blades of seedlings furnished carbon dioxide but no nitrates weighed 14.5 percent less than those of seedlings grown on their own reserves of both carbon and nitrogen (table 4). Leaf blades of the seedlings given carbon dioxide had an average length of 11.1 cm., whereas those not given carbon dioxide had a length of 13.5 cm. A gain of 150 percent in the green weight of the leaf blades resulted when extra nitrogen but no extra carbon dioxide was furnished. In table 5 it is shown that seedlings grown without both extra nitrogen and carbon dioxide produced 49 grams of fresh tissue per 0.1 gram of reserve nitrogen and 88 grams when extra nitrogen only was supplied. Similar seedlings grown with extra carbon dioxide but without extra nitrogen yielded 47 grams of tissue. Seedlings to which both carbon dioxide and nitrates were furnished produced 97 grams of tissue, a gain of only 9 grams by the use of carbon dioxide; indicating that the carbohydrate reserves of the seed were sufficient to allow the utilization of considerable additional nitrogen. The principal effect of supplying extra carbon dioxide but no nitrates was in stunting of the leaves whereas the chief effect of

supplying nitrates but no carbon dioxide was in increasing the weight, size, and number of leaves.

### Illinois High-protein Corn

In comparison to the other types of seedlings, the green weight of the high-protein corn seedlings was influenced relatively little by differences in external environment during the ten-day period of growth. Internal differences in the contents of the cells and anatomical structure were observed, however. Growth was increased somewhat more by additional carbon dioxide than by nitrates. The average weight of a seedling grown on its own reserves of both carbon and nitrogen was 2.46 grams whereas that of a seedling given extra carbon dioxide but no nitrates was 2.85 grams and that of seedling supplied with nitrates but no extra carbon dioxide was 2.62 grams. When given both additional nitrogen and carbon dioxide the average weight was 3.03 grams. From the data presented in table 4, it may be noted that the presence of carbon dioxide in the atmosphere surrounding the seedlings did not modify the amount or type of growth to nearly so great an extent as it did with seedlings grown from higher protein types of seeds. In table 4, it is also shown that nitrates influence the amount and kind of growth of low-protein corn seedlings more than that of the high-protein corn seedlings. This is perhaps because the store of reserve nitrogen in proportion to the carbohydrates is much greater in the high-protein corn. The growth of roots of the high-protein corn seedlings was not appreciably influenced by supplying either carbon dioxide or nitrates, although there was a slight increase when both nitrates and carbon dioxide were furnished.

### New Era Cow Pea

The seeds from which these seedlings were grown had a high nitrogen (4.4 percent) and a high starch content. In tables 1 and 2 it may be observed that the seedlings gained more in weight by the use of carbon dioxide than they did by the use of nitrates. The average weight of a seedling grown without addition of carbon dioxide or nitrates was 0.84 grams whereas that of a seedling given carbon dioxide but no nitrates was 2.05 grams and that of one furnished nitrates but no carbon dioxide was 1.17 grams. The average weight of a seedling given both nitrates and carbon dioxide was 3.28 grams. In table 4 it is shown that a gain of 243 percent in green weight of roots, of 112 percent in the green weight of stems, and of 103 percent in the green weight of leaves resulted by supplying extra carbon dioxide but no nitrates whereas a gain of 100 percent in weight of roots and only a slight gain in weight of shoots resulted from supplying nitrates but no carbon dioxide. Both hypocotyls and roots of the seedlings receiving nitrates were larger in diameter than those of unnitrated seedlings. The 100-percent gain in the fresh weight of roots with the use of nitrates was brought about chiefly by the increase in the root diameters. There was no apparent

increase in number or length of roots. The carbon-dioxid-treated seedlings, however, had remarkable increases in number and length of roots as well as in weight as compared to seedlings which did not receive carbon dioxide.

The blades of the leaves did not vary greatly in size with differences in external conditions. Cow pea seedlings of this variety quickly utilize the reserves in the cotyledons and form a set of leaves which are relatively thick in cross section and have a very compact structure. The second set of leaves is compound. Much of the nitrogenous food reserve is utilized in forming the first set of relatively large leaves. Because of the compact cellular structure of these first leaves and of their high protoplasmic but low water content, the cow pea seedling appears to produce a smaller amount of leaf tissue per unit of reserve nitrogen (table 5, column 12) than the other types of seedlings which were used. In table 1, column 9, it may be noted that the weight of leaves of the plants synthesizing carbohydrates is much higher than that of plants grown in the atmosphere lacking carbon dioxide. This difference in weight is partially due to the very high starch content of the leaves receiving carbon dioxide (see photographs, 10).

### Peking Soy Bean

The non-nitrogenous carbon compounds of this seed are stored largely in the form of fats, although considerable starch is also present. The seed contains 3.4 percent nitrogen. The seedlings gained more in size and weight by the utilization of carbon dioxide than by that of nitrates. Figures 1 to 4 in Plate LXVIII illustrate the appearance of soy bean seedlings under the four sets of environmental conditions. In tables 1 and 2 it is shown that the average weight of a seedling grown without the addition of carbon dioxide or of nitrates was 0.75 grams whereas when supplied with carbon dioxide but no nitrates it was 1.48 grams, and when given nitrates but no carbon dioxide was 1.00 gram. When both nitrates and carbon dioxide were given, the average weight was 1.68 grams. Table 4 shows that the percentage gain in fresh weight of roots with carbon dioxide supplied is 249, with nitrates supplied it is 100, and with both nitrates and carbon dioxide it is 220. The stems gained 59 percent with carbon dioxide supplied but did not gain when nitrates but no carbon dioxide were given. The leaves increased appreciably in weight with the addition of carbon dioxide but not with nitrates unless carbon dioxide was also furnished. As was the case with cow pea seedlings, the roots of the nitrated plants had larger diameters than those of the unnitrated plants. The gain in weight of roots with the use of nitrates is brought about partially by the increase in diameter of the roots. The roots of the plant receiving nitrates but not carbon dioxide were somewhat longer but not much more numerous (Pl. LXVIII, fig. 3) than those of the corresponding plant which did not receive nitrates (fig. 1). Roots of a seedling furnished carbon dioxide are much longer and much more numerous (fig. 2) than those of a seedling not given carbon dioxide. The addition of



both nitrates and carbon dioxid (fig. 4) resulted in an increase in the weight of stems and leaves.

### **Red Rocky Ford Muskmelon**

These seeds have the highest nitrogen content (5.62 percent) of all the types used in these experiments. The non-nitrogenous reserves are stored chiefly in the form of fats. Like the other kinds of seedlings grown from seeds with a high nitrogen content, there was a greater gain in weight with the use of carbon dioxid than with that of nitrates. The average weight of a seedling grown without addition of carbon dioxid or nitrates was 0.292 grams whereas when grown with the addition of carbon dioxid but no nitrates it was 0.726 grams and seedlings to which nitrates but no carbon dioxid was given averaged only 0.384 grams. When both nitrates and carbon dioxid were supplied the average weight was 1.45 grams (tables 1 and 2, column 11). In table 5 it is shown that muskmelon seedlings grown without addition of carbon dioxid or nitrates produced 39 grams of fresh tissue per 0.1 gram of reserve nitrogen but 97 grams if carbon dioxid only was supplied. Seedlings to which nitrate nitrogen but no carbon dioxid was given yielded 52 grams of fresh tissue and those furnished carbon dioxid as well as nitrates yielded 194 grams.

The roots gained 292 percent (table 4) when carbon dioxid only was supplied but only 33 percent when extra nitrogen without carbon dioxid was given. When both extra nitrogen and carbon dioxid were furnished the roots gained 570 percent over the checks grown without either carbon dioxid or nitrates. The synthesis of carbohydrates resulted in a very great increase in number and length as well as in weight of roots. The roots of the nitrated seedlings tended to be coarser than those of the unnitrated plants. The stems and hypocotyls gained appreciably in weight with the use of additional carbon dioxid and without nitrates but only slightly by the use of additional nitrates unless carbon dioxid was also supplied. Seedlings not given carbon dioxid did not develop leaves to the stage of unfolding unless extra nitrogen was obtainable. Seedlings furnished carbon dioxid but no additional nitrogen developed large cotyledons but only a few of these seedlings developed true foliage leaves and these were very small. Plants to which both carbon dioxid and nitrates were supplied produced two relatively large foliage leaves with the third leaf nearly ready to unfold.

### **Mammoth Russian Sunflower**

The food reserves of these seeds contained much oil and a relatively large amount of nitrogen (4.56 percent). The average weight of a seedling grown without addition of either carbon dioxid or nitrogen was 0.835 grams, whereas that of a seedling receiving carbon dioxid but no nitrates was 1.69 grams and that of one receiving nitrates but no additional carbon dioxid was 0.752 grams. The average weight of a seedling receiving both nitrates and carbon dioxid was only 1.77 grams (tables 1 and 2, column 11). The results

summarized in table 4 show that the addition of nitrates without carbon dioxide to the seedlings did not appreciably increase or modify the type of growth but that the addition of carbon dioxide without nitrates greatly increased the growth and especially that of the roots. The green weight of roots was increased 194 percent when carbon dioxide only was supplied but only 14 percent when nitrates only were given. They gained 180 percent when both nitrates and carbon dioxide were furnished. Seedlings receiving carbon dioxide produced adventitious roots at the bases of the hypocotyls but none were found on seedlings not given carbon dioxide. The stems and hypocotyls gained considerably in weight with the use of carbon dioxide. Photographs of sunflower seedlings grown under the four sets of environmental conditions are shown in Plate LXVIII, figures 5 to 8.

### General Observations on Growth of the Various Types of Seedlings

Seedlings grown from seeds having a large reserve of starch but a small amount of nitrogen are greatly benefited by extra nitrogen in the form of nitrates but are not benefited and may even show injury by the use of extra carbon in the form of carbon dioxide unless extra nitrogen is also given. Seeds having a large reserve of nitrogen but somewhat limited amounts of non-nitrogenous carbon compounds produce seedlings which respond favorably to additional carbon dioxide but much less to additional nitrogen.

This relation of the relative amounts of carbohydrates and nitrogen to growth was found in all the types of seedlings which were used and is in agreement with results reported by Kraus and Kraybill (6).

The utilization of extra carbon in the form of carbon dioxide and extra nitrogen in the form of nitrates have different effects on the relative amounts of growth of the various organs. In general, the synthesis of carbohydrates results in a comparatively greater increase in the growth of roots and stems, particularly of roots than of leaves, unless extra nitrogen is also given. If the carbohydrate reserves of the seed are sufficient to permit the utilization of additional nitrogen, as was true with low-protein corn seedlings, the extra nitrogen tends to cause a relatively greater increase in the growth of leaves than of roots. If seedlings are supplied with both carbon dioxide and nitrates, the proportions of shoots to roots appear to be affected by the relative rates of synthesis of nitrogenous and carbohydrate compounds. In order definitely to determine the situation with respect to the influence of the relative rates of synthesis of carbohydrate and nitrogen compounds on the growth of shoots and roots, more exact quantitative chemical methods will be required.

### Shoot-to-root Ratios

The ratios of green weights of shoots to roots do not necessarily give a correct impression of the relative amounts and kinds of growth of shoots and roots under the various environmental conditions. Judging the type of growth by green weights only may furnish a somewhat misleading picture, as

for example, the roots of several types of the nitrated plants tended to be coarser but not noticeably or appreciably more numerous than those of corresponding plants grown without nitrates. It is highly important to consider the number of roots initiated as well as their length, diameter, and weight. Under certain conditions a plant may produce a relatively extensive system of finely branching roots which may weigh considerably less than those of another plant which are coarser, shorter, and much less profusely branched. Volume measurements in such cases are of no greater value than are the fresh weights. Photographs may afford a more accurate conception of the relative differences in growth of roots under varying conditions of environment than do the fresh weights.

#### **Microchemical Evidence of the Effect of the Different Conditions for Growth on the Chemical Composition of the Seedlings**

Seedlings of high-protein corn grown both with and without nitrates contained somewhat less starch but approximately the same amount of free-reducing substances when grown without as with carbon dioxid. No marked differences in chemical composition of these seedlings resulting from the presence or absence of nitrates and the presence or absence of carbon dioxid were found at the end of the nine-day period in which these seedlings were growing.

Seedlings of low-protein corn not supplied with nitrates and carbon dioxid contained less starch but approximately the same amount of reducing substances as similar seedlings which received carbon dioxid. Seedlings which received both nitrates and carbon dioxid contained slightly more carbohydrates than similar seedlings which did not receive carbon dioxid. The presence or absence of nitrates in the nutrient medium affected the supply of reserve carbohydrates in the low-protein seedling much more than the presence or absence of carbon dioxid in the atmosphere.

Cow pea and soy bean seedlings which received carbon dioxid contained much more starch and free-reducing substances than similar seedlings which were not permitted to synthesize carbohydrates. Plants receiving nitrates and carbon dioxid had much less starch and free-reducing substances than similar plants not receiving nitrates.

The roots, hypocotyls, stems, and cotyledons of muskmelon seedlings grown in the atmosphere containing 0.4 percent carbon dioxid were gorged with starch. More carbohydrates were produced than by any other kind of seedling used in these experiments. It has been found in other experiments that tomato seedlings similarly have an unusual capacity for the rapid synthesis of both carbon dioxid and inorganic nitrogen into products which promote growth. Carbohydrates tend to accumulate very rapidly in these two kinds of seedlings if the nitrogen supply is somewhat limited. Only a few starch grains and a small amount of reducing substances were found in sections of tissues of seedlings grown in the atmosphere to which no carbon dioxid was supplied.

Sunflower seedlings receiving carbon dioxide contained very small amounts of starch, relatively more of reducing substances, and very large amounts of fat. The tissues of seedlings grown without carbon dioxide contained very small amounts of carbohydrates. Starch was present only in the guard cells of the leaves. Only slight traces of reducing substances were found in the tissues of the different organs but some fat was found particularly in the cotyledons.

It appears from the results shown in tables 3 and 4 that seedlings grown from seeds having a high protein and high oil content synthesize carbohydrates more rapidly in the early phases of growth than seedlings of low-protein seeds. The high-protein, high-oil seeds have their food reserves stored in tissues that become foliage organs, the plastids of which are capable of functioning in carbohydrate synthesis very early in the growth of the seedling. It takes a considerably longer time for other types such as corn to develop their foliage tissues to the stage at which an appreciably extensive synthesis of carbohydrates may occur. Doubtless other unknown factors have also influenced the amount of carbohydrate synthesis in the young seedlings which have been used in these investigations.

The accumulation of greater amounts of carbohydrates or fats in the tissues of seedlings grown without nitrates as compared with the amounts found in seedlings to which nitrates were supplied is in accord with observations of Kraus and Kraybill (6) and also of other investigators and is to be attributed to the fact that both synthesis of organic nitrogen compounds and utilization of carbohydrates in growth are limited by the lack of nitrogen. When carbohydrate synthesis is limited there is less of free-reducing substances, starch, and cell membrane substances in seedlings grown with nitrates than without nitrates, since part of the reserve carbon must be used in the synthesis of organic nitrogen compounds from nitrates.

Rather definite correlations have been found between the relative amounts of carbohydrates and fats which accumulate in the tissues of the seedlings under different conditions and the number and weight of roots produced. The high-protein types of seedlings—soy bean, cow pea, muskmelon, and sunflower—when grown in the atmosphere containing carbon dioxide accumulate large amounts of reserve carbohydrates in their tissues and at the same time develop relatively large root systems. Similar seedlings grown without carbon dioxide have very little reserve carbohydrate in their tissues and have comparatively small root systems. Seedlings of high- and low-protein corn which did not gain much in green weight due to the utilization of carbon dioxide had relatively small differences in the carbohydrate content of their tissues. It was also found that there were no marked differences in the number and weight of roots produced by corn seedlings receiving carbon dioxide as compared with others which did not receive it. The carbohydrate reserves of the seed especially of the low protein type were sufficient to enable the plants to produce root systems of

approximately the maximum size and weight characteristic for seedlings of these kinds.

### Effect of Conditions Influencing the Supply of Available Nitrogen and Carbohydrates Upon the Growth Response as to Thickness of Tissue, Lignification, etc.

Microscopic examination of the tissues of the various types of seedlings has definitely indicated the effect of different amounts of carbohydrates on the development of strengthening tissues. Seedlings which synthesized an abundance of carbohydrates had thicker walled xylem vessels and apparently much more extensive lignification of bast fibers than seedlings grown under conditions which prevented access to extra carbon. Less difference of the effect of synthesizing or not synthesizing carbohydrates was noted in the case of low-protein corn seedlings than in those of high-protein types. This variation in response is undoubtedly related to the difference in the relative amounts of reserve carbon and nitrogen in the seeds.

Concerning the direct effect of light on the development of strengthening tissues only an incomplete report can be made. The cells of low-protein corn seedlings used in other experiments and grown in darkness appeared to have about as much thickening of the walls as was found in similar seedlings grown in the light in the carbon-dioxid-free atmosphere. Seedlings of higher protein seeds grown in light and without carbon dioxid had slightly more thickening of walls than was found in similar seedlings grown in darkness. It seems possible that because of the apparently greater ability of these seedlings than of the low-protein types to synthesize carbohydrates in their early growth that some of the carbon dioxid given off in respiration may have been synthesized into compounds which were used in producing the slightly greater thickening of the cell walls.

## DISCUSSION

### Growth of Roots

In table 6 it may be noted that soy bean and cow pea seedlings develop

TABLE 6. *Weight of Roots in Grams per Plant Produced by Seedlings Grown Without Extra Nitrogen, Some in Darkness, and Others in Light, With or Without Carbon Dioxid in the Atmosphere*

	Darkness * Normal Atmosphere	Light - CO <sub>2</sub>	Light + CO <sub>2</sub>
High-protein corn .....	0.686	1.140	1.155
Low-protein corn .....	0.750	1.080	0.991
New Era cow pea .....	0.237	0.220	0.756
Peking soy bean .....	0.172	0.183	0.640
Red Rocky Ford muskmelon .....	0.040	0.089	0.349
Mammoth Russian sunflower .....	0.105	0.157	0.462

\* The experiment for which these seedlings were grown was described in a previous publication (11).

TABLE 7. *Lengths and Fresh Weights of Stems plus Hypocotyls Attained per Plant by Seedlings Grown Without Extra Nitrogen, Some in Darkness, and Others in Light, With or Without Carbon Dioxid in the Atmosphere*

	Darkness* Normal Atmosphere		Light - CO <sub>2</sub>		Light + CO <sub>2</sub>	
	Length cm.	Weight gms.	Length cm.	Weight gms.	Length cm.	Weight gms.
High-protein corn.....	36.0	0.774	25.7	0.529	28.6	0.792
Low-protein corn.....	29.2	0.715	20.2	0.494	17.7	0.584
New Era cow pea.....	32.1	0.699	10.9	0.344	16.4	0.731
Peking soy bean.....	40.0	0.568	11.6	0.244	17.0	0.388
Red Rocky Ford muskmelon.	14.0	0.201	3.4	0.058	4.0	0.133
Mammoth Russian sunflower	27.2	0.855	12.3	0.393	18.1	0.818

\* A description of these seedlings and the experimental methods was given in a previous publication (11).

practically the same weight of roots in darkness and in light in an atmosphere not supplied with carbon dioxid. Muskmelon and sunflower seedlings produce a greater weight of roots in the light but this may be partially accounted for by the fact that in the light they doubtless made use of some of the respired carbon dioxid. High- and low-protein corn seedlings developed considerably more roots in light than in darkness. The seedlings grown in darkness were exposed to a temperature approximately 4 degrees lower than that used for the seedlings grown in the light, which may be responsible to some extent for the observed differences in the amount of roots produced under the two conditions. Other lots of corn seedlings grown in darkness at higher temperatures than were used for the seedlings mentioned in table 6 had considerably larger root systems. There is also the possibility that light favored the utilization of carbohydrates in the growth of roots. To answer this question somewhat more definitely, it will be necessary to place seedlings in nutrient solutions containing a soluble form of carbohydrate such as glucose and to keep some of them in darkness and others in light in an atmosphere lacking carbon dioxid. In this way it may be possible to determine if light in itself affects the utilization of carbohydrates in the promotion of growth of roots.

The question arises as to what types of non-nitrogenous carbon compounds favor rooting. It has been noted that certain seedlings such as cow pea and muskmelon store up large amounts of starch and reducing substances, and that growth of roots is greatly fostered during the process of this accretion of carbohydrate materials. Other seedlings such as sunflower have their carbon reserves accumulate chiefly in the form of fats and during the time in which this accretion of material occurs, roots grow profusely. Since there is a conspicuous gain in growth of roots with the accumulation of carbon compounds in the form of fats as well as in the form of starch, the type of compound more directly concerned in the growth of roots must be a

simpler and a soluble type of compound common to both the starch- and fat-forming plants. During the process of carbohydrate or fat accumulation a disposition for rapid growth and initiation of roots is established. Chemical changes of importance in the growth of roots, other than the formation and accumulation of carbohydrates, must doubtless also occur at this time.

### Growth of Stems and Hypocotyls

The measurements of lengths of stems and hypocotyls furnish a better picture of differences in type of growth under different external conditions than do the weights. This is partly because the stem tissues of seedlings synthesizing carbohydrates are more compact, are heavier walled, and contain more storage carbohydrates. A given length of stem of a seedling grown in the light in an atmosphere containing carbon dioxide tends to weigh more than that of a similar seedling grown in darkness.

Growth of the stem and hypocotyl is favored by having an abundance of carbohydrates since stems and hypocotyls of seedlings synthesizing carbohydrates were longer and heavier than those of plants in which photosynthesis was restricted. Low-protein corn seedlings were an exception in this respect. Seedlings grown from seeds having a high nitrogen content have available less non-nitrogenous carbon compounds than are necessary to allow the greatest amount of growth from the reserve nitrogen. It is also shown and in agreement with results of Godlewski (3) that light in itself has an inhibiting action on the growth in length of hypocotyls and stems since seedlings grown in light in an atmosphere not supplied with carbon dioxide are shorter and weigh much less than those of seedlings grown in darkness. Godlewski's experiments showed also that a greater amount of the total dry matter of the seedling grown in darkness went into the hypocotyl and stem than it did in the seedling grown in the light.

### Growth of the Leaves and Foliaceous Cotyledons

The data presented in table 8 show that light influences the growth of leaves and foliaceous cotyledons in other ways than by the synthesis of

TABLE 8. *Weight in Grams of Foliaceous Tissue Produced per Plant by Seedlings Grown Without Extra Nitrogen, Some in Darkness, and Others in Light, With or Without Carbon Dioxide in the Atmosphere*

	Darkness* Normal Atmosphere	Light - CO <sub>2</sub>	Light + CO <sub>2</sub>
High-protein corn . . . . .	0.696	0.791	0.902
Low-protein corn . . . . .	0.575	0.516	0.441
New Era Cow Pea . . . . .	0.058	0.276	0.561
Peking soy bean . . . . .	0.074	0.321	0.451
Red Rocky Ford muskmelon . . . . .	0.040	0.145	0.244
Mammoth Russian sunflower . . . . .	0.165	0.285	0.410

\* A description of these seedlings was given in a previous publication (11).

carbohydrates. Seedlings grown in the light under conditions which inhibited carbohydrate synthesis had larger leaves and cotyledons which also weighed more than those of seedlings grown in darkness. The only exception to this mode of behavior was that of the leaves of low-protein corn seedlings and in these the differences were not great.

The leaves and cotyledons of seedlings grown without nitrates from high-protein types of seeds are benefited by the synthesis of carbohydrates whereas those of the lower protein types are smaller and weigh less if carbon dioxide is supplied than if it is not furnished. Not only is there this difference in size and weight of leaves under the two conditions but the leaves are greener if the plants are not permitted to utilize additional carbon as carbon dioxide. Similar experiments with wheat seedlings grown from seeds of relatively low-nitrogen, high-carbohydrate content produced the same response as to size and greenness. The leaves and cotyledons of the high-protein types of seedlings grown also without nitrates tend to be greener in the early phases of growth if extra carbon is supplied. It might be expected, however, that if similar experiments were conducted with seedlings grown in a series having varying concentrations of carbon dioxide in the atmosphere, certain concentrations might lead to the synthesis of just enough additional carbohydrates so that these seedlings would have available the same amount of carbohydrate compounds in relation to the stored nitrogen that low-protein corn seedlings had as a natural endowment and that the growth responses of these seedlings would be somewhat like those of low-protein corn. More recent experiments with squash seedlings tend to give some support to this hypothesis. The present indications are that a very large or very small amount of available carbon in proportion to nitrogen produce internal conditions unfavorable to the development of a healthy green color and of relatively large size of leaves.

Nightingale (7) reported an increase in greenness of leaves of tomato plants having a large amount of carbohydrate but low nitrogen reserves, on changing the plants from a 14-hour to 6-hour exposure to June sunlight. The factors involved in inducing the greater greenness of leaves in the short day may be related to those in the experiments described above.

#### SUMMARY

1. (a) Seedlings grown from seeds having a high carbohydrate but low nitrogen reserve have their growth increased more by the use of nitrates than they do by the use of carbon dioxide.

(b) Seedlings grown from seeds having a relatively low carbohydrate but high nitrogen content have their growth increased more by the use of carbon dioxide than they do by the use of nitrates.

(c) If light is not a limiting factor most types of seedlings grow most rapidly when supplied with both carbon dioxide and nitrates, especially during the later phases of seedling development.



2. The synthesis of carbohydrates by seedlings results in a relatively greater increase in growth of roots and stems, particularly of roots, than of leaves unless extra nitrogen is also given.

3. The utilization of nitrates causes a relatively greater increase in growth of leaves and stems than of roots.

4. Seedlings grown from seeds containing relatively large amounts of carbohydrates in proportion to nitrogen do not synthesize carbohydrates extensively unless extra nitrogen is furnished. These seedlings correspondingly do not increase the weight and size of their roots when grown in an atmosphere containing carbon dioxide as do the high-protein seedlings which synthesize carbohydrates abundantly.

5. Seedlings grown from seeds containing relatively large amounts of nitrogen in proportion to carbohydrates do not have the capacity to utilize nitrates extensively unless carbon dioxide is also furnished. Correspondingly the effect of nitrates in stimulating the production of leaf tissue does not occur to so great an extent as in the case of seedlings grown from low-protein seeds.

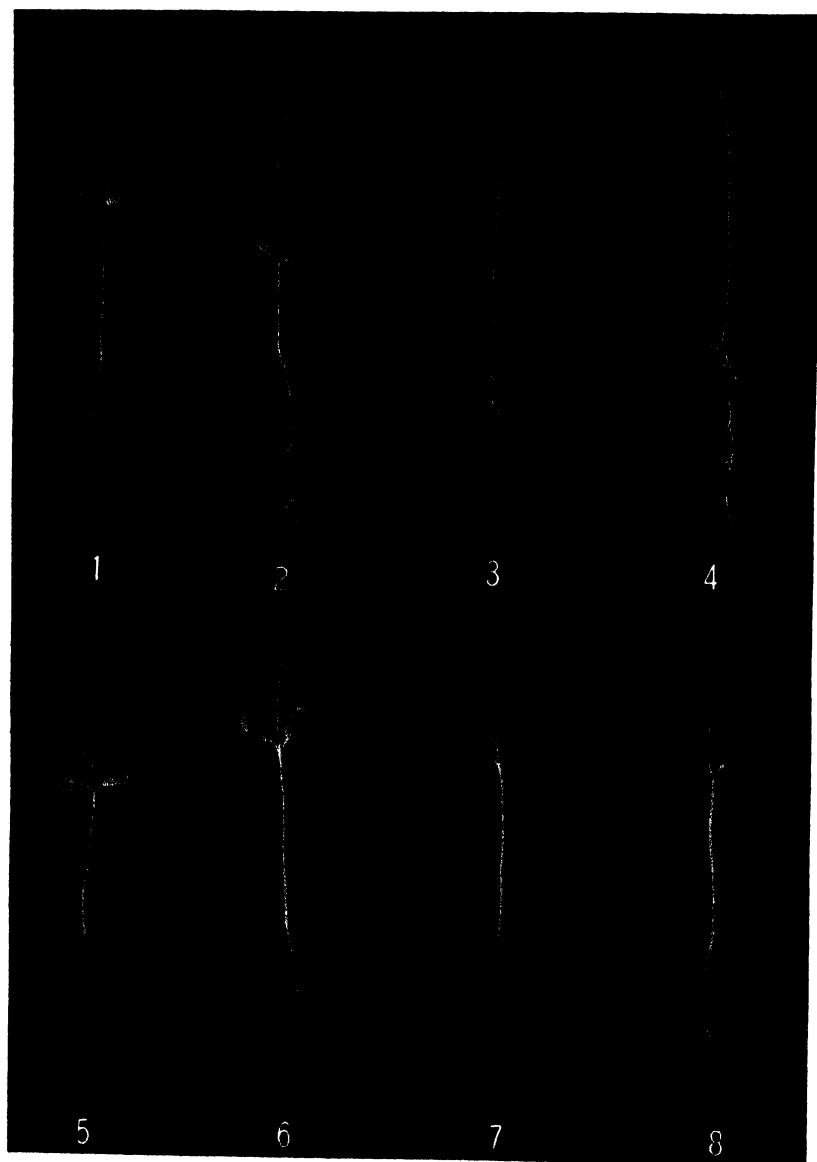
6. Seedlings grown without extra nitrogen accumulate reserve carbohydrates more rapidly than those supplied with nitrates.

7. Seedlings which synthesized an abundance of carbohydrates and especially those which accumulated large amounts of carbohydrates in the cells had thicker walled xylem vessels and greater amounts of lignification of bast fibers than seedlings grown under conditions which prevented access to extra carbon dioxide.

8. It is shown that exposure to light during the normal length of days in May and June produces an inhibiting effect on the growth in length of the stem and hypocotyl but a stimulating effect on the growth of leaves and foliaceous cotyledons.

9. Other investigators have stated that the synthesis of carbohydrates has no direct effect on the growth of leaves. The results of these seedling experiments have shown that the synthesis of relatively large amounts of carbohydrates has a growth-limiting and a chlorophyll-deficiency effect on leaves unless a supply of available nitrogen is maintained during the synthesizing process.

These investigations were conducted at the Boyce Thompson Institute for Plant Research from 1924 to 1926. I wish to thank Dr. Ward B. Davis for making determinations of the carbon dioxide content of the atmosphere in the culture chambers; also the Department of Plant Genetics of the University of Illinois for supplying the seeds of high- and low-protein corn.



REID: GROWTH



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## EXPLANATION OF PLATE LXVIII

FIG. 1. Peking soy bean seedling grown without nitrates and without carbon dioxide.

FIG. 2. Peking soy bean seedling grown without nitrates and with carbon dioxide.

FIG. 3. Peking soy bean seedling grown with nitrates and without carbon dioxide.

FIG. 4. Peking soy bean seedling grown with nitrates and with carbon dioxide.

FIG. 5. Mammoth Russian sunflower seedling grown without nitrates and without carbon dioxide.

FIG. 6. Mammoth Russian sunflower seedling grown without nitrates and with carbon dioxide.

FIG. 7. Mammoth Russian sunflower seedling grown with nitrates and without carbon dioxide.

FIG. 8. Mammoth Russian sunflower seedling grown with nitrates and with carbon dioxide.

## EFFECT OF VARIATIONS IN THE AMOUNTS OF AVAILABLE CARBON AND NITROGEN ON THE GROWTH OF WHEAT SEEDLINGS

MARY E. REID

A study is here reported of the development under different external conditions of wheat seedlings from seeds having varying proportions of carbohydrates and nitrogen.

### EXPERIMENT I. APRIL 24-MAY 6, 1926

Wheat seedlings grown from three different types of wheat were used:

	Weight, gms.	Total Nitrogen, %	Amount of Nitrogen, mgs.
Marquis, Dickinson, N. D.....	2.94	2.37	59.0
Marquis, Davis, Calif.....	2.60	1.45	37.7
Little Club, Davis, Calif.....	2.66	1.52	40.4

The seeds were sterilized by immersion in a 0.25-percent solution of Uspulun for one hour, and were placed in germinators between layers of moist filter paper April 24 and were planted in sterilized quartz sand (No. 3) in 7-inch bulb pots April 26 (1). There were four cultures of each of three types of wheat. Two of each of the three sets of cultures were given the nutrient solution containing nitrates, and two of each set the solution lacking nitrates. The cultures were placed in the bell jar apparatus described in the preceding paper (2) and were sealed air tight with DeKhotinsky cement. One of each of the pairs of cultures was given approximately 0.4 percent carbon dioxid in the incoming air and the other was placed in a chamber receiving air lacking carbon dioxid. A reduced pressure equal to 0.004 atmospheres was maintained for all of the cultures. Fresh nutrient solutions were given every third day and the moisture content of the sand was maintained during intervening times by the addition of freshly distilled water. The cultures were removed from the apparatus May 6 and were immediately placed in darkened moist chambers until the seedlings could be weighed and measured. The temperature and light conditions were both highly favorable for rapid growth. The days were all clear except two which were only partly cloudy.

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## EXPERIMENT 2. MAY 10-22, 1926

Although the Marquis wheats used in experiment 1 were of the same strain, it was considered desirable to study the effects of high- and low-protein contents of wheat which had been grown under similar environmental conditions. In this experiment hard and soft grains were selected from the Marquis wheat grown at Davis, California. The Marquis wheat from Dickinson, N. D., was also used.

	Weight of Grains, gms.	Total Nitrogen, %	Amount of Nitrogen, mgs.
Marquis, Dickinson, N. D. ....	2.12	2.37	50.2
Marquis, Davis, Calif. (Hard grains)....	2.33	2.11	49.1
Marquis, Davis, Calif. (Soft grains)....	2.21	1.45	32.2

Four cultures of each type of wheat were grown and the same conditions for growth were employed as in experiment 1. Due to the fact that the season was somewhat more advanced and the temperature was high, a canopy of cheesecloth was stretched across the greenhouse about thirty inches above the bell jars for four or five hours during the middle of the day on six of the ten days during which the seedlings were growing. The temperature and light conditions were, therefore, slightly different in experiments 1 and 2.

## DATA

The results show that growth of the seedling is markedly influenced by changes in the external environment with respect to the available supply of carbon and nitrogen and also by differences in the amounts of nitrogen and carbohydrates stored within the seeds. The relative amount of reserve carbohydrates is of particular importance if the seedlings are grown under conditions which limit the synthesis of these substances from carbon dioxide and the relative amount of reserve nitrogen is likewise of particular importance if the seedlings are grown under conditions which limit or inhibit the intake of additional nitrogen.

The utilization of carbon dioxide resulted in a greater total weight of green and dry tissue whether the seedlings were grown with or without additional nitrogen. If nitrates were not supplied, additional carbon dioxide favored the growth of roots much more than that of shoots and in most cases the roots of high-protein seedlings had their growth increased by carbon dioxide somewhat more than those of low-protein seedlings. The addition of nitrates to the carbon-dioxide-treated seedlings promoted the growth of shoots more than that of roots and the growth of shoots of low-protein seedlings considerably more than that of high-protein seedlings.

The green weights of shoots and roots of experiment 1 are shown in table 1 and those of experiment 2 in table 2. The high-protein Marquis wheat

seedlings grown without nitrates in experiment I produced 12.5 grams of shoots and 9.9 grams of roots with added carbon dioxide and 12.1 grams of shoots and 6.8 grams of roots without the addition of carbon dioxide. The low-protein Marquis wheat seedlings of the same experiment yielded 8.9 grams of shoots and 9.6 grams of roots when additional carbon dioxide was

TABLE 1. *Wheat of High- and Low-protein Strains Grown With and Without Addition of Carbon Dioxide, and With and Without Nitrates, April 24-May 6, 1926*

Without Nitrates (88 Plants)

Type of Wheat	Total Nitrogen in Seed %	CO <sub>2</sub> in Air Entering Chamber	Green Weights		Dry Weights		Percentage of Dry Matter		Ratio of Green Weight of Shoots to Roots
			Shoots	Roots	Shoots	Roots	Shoots	Roots	
Marquis.....	2.37	0.4%	12.47	9.90	1.51	0.85	12.2	9.1	1.26
Marquis.....	2.37	none	12.07	6.82	1.26	0.43	10.5	6.4	1.77
Marquis.....	1.45	0.4%	8.90	9.64	1.47	0.92	16.6	9.5	0.92
Marquis.....	1.45	none	9.05	7.42	1.01	0.64	12.2	8.7	1.22
Little Club....	1.52	0.4%	9.38	10.87	1.41	0.92	15.1	8.5	0.86
Little Club....	1.52	none	10.54	8.47	1.21	0.67	11.5	8.0	1.24

With Nitrates

Marquis.....	2.37	0.4%	16.42	11.48	1.74	0.86	10.6	7.6	1.43
Marquis.....	2.37	none	11.63	6.97	1.18	0.54	10.2	7.7	1.66
Marquis.....	1.45	0.4%	16.96	12.67	1.90	1.00	11.2	7.9	1.34
Marquis.....	1.45	none	8.36	7.04	0.94	0.53	11.3	7.6	1.18
Little Club....	1.52	0.4%	16.80	12.82	1.84	0.98	10.5	7.7	1.31
Little Club....	1.52	none	8.39	7.50	0.83	0.47	10.0	6.3	1.12

TABLE 2. *Marquis Wheat of High- and Low-protein Strains Grown With and Without Addition of Carbon Dioxide, and With and Without Nitrates, May 10-22, 1926*

Without Nitrates (75 Plants)

Type of Wheat	Total Nitrogen in Seed %	CO <sub>2</sub> in Air Entering Chamber	Green Weights		Dry Weights		Percentage of Dry Matter		Ratio of Green Weights of Shoots to Roots
			Shoots	Roots	Shoots	Roots	Shoots	Roots	
Dickinson, N. D..	2.37	0.4%	9.64	7.08	1.30	0.48	13.6	5.8	1.22
Dickinson, N. D..	2.37	none	7.83	4.01	0.83	0.33	10.6	8.3	1.95
Davis, Calif.....	2.11	0.4%	9.72	7.25	1.22	0.63	12.6	8.7	1.34
Davis, Calif.....	2.11	none	9.25	5.32	1.01	0.43	11.0	9.1	1.73
Davis, Calif.....	1.45	0.4%	7.44	7.60	1.19	0.72	16.1	9.5	0.98
Davis, Calif.....	1.45	none	6.96	5.14	0.78	0.51	11.3	10.0	1.35

With Nitrates

Dickinson, N. D..	2.37	0.4%	14.02	8.40	1.64	0.64	9.4	6.1	1.66
Dickinson, N. D..	2.37	none	11.80	5.14	1.10	0.39	9.3	7.7	2.29
Davis, Calif.....	2.11	0.4%	15.20	9.12	1.71	0.75	11.3	8.3	1.66
Davis, Calif.....	2.11	none	12.11	6.00	1.07	0.45	8.9	7.6	2.02
Davis, Calif.....	1.45	0.4%	12.99	8.60	1.55	0.71	12.0	8.2	1.51
Davis, Calif.....	1.45	none	10.50	4.98	0.96	0.38	9.2	7.8	2.10

given and 9.1 grams of shoots and 7.4 grams of roots when carbon dioxide was withheld. The data presented in tables 1 and 2 have been summarized in table 5 which shows the percentage gain or loss over the checks due to the addition of carbon dioxide. In column 6 of this table it may be noted that the increases in green weights of roots of unnitrated seedlings resulting from the use of carbon dioxide varied from 28 percent with a low-protein wheat to 76 percent with a high-protein wheat. In two of the three lots of seedlings grown from low-protein grains there was a slight decrease in the green weight of the shoots. In column 5 of table 5 the effect of added carbon dioxide upon the growth of shoots may be observed. The slight decreases in green weights of shoots of the unnitrated seedlings furnished with carbon dioxide as compared to those not given carbon dioxide would not be considered significant were it not for the fact that there were conspicuous differences in the appearance of the shoots of the two sets of seedlings, the shoots of the seedlings not receiving carbon dioxide being longer. The leaf blades of the high-protein Marquis wheat seedlings of experiment 1, grown without added carbon dioxide, had an average length of 15.9 cm. whereas those of similar seedlings which received carbon dioxide had an average length of 13.3 cm. Leaf blades of low-protein seedlings of the same variety and strain averaged 10.8 cm. in length when grown without added carbon dioxide and 10.2 cm. when extra carbon dioxide was given. With the exception of one set of low-protein seedlings, similar differences in length of leaf blades with carbon dioxide added or withheld were found in experiment 2. In all cases the differences in length between leaf blades of high-protein seedlings grown with and without added carbon dioxide were greater than those of corresponding low-protein seedlings. The roots of unnitrated seedlings receiving carbon dioxide, especially those of the high-protein types, were heavier, somewhat longer, and considerably more branched than those of seedlings not supplied with carbon dioxide. With a greater amount of stored nitrogen in the wheat grains, there were greater differences in growth of both shoots and roots due to the addition of carbon dioxide to the atmosphere.

The relative sizes of shoots and roots and the general appearance of the plants may be observed in the illustrations of Plate LXIX. The unnitrated high-protein Marquis wheat seedlings grown without carbon dioxide are shown in figure 1 and those given carbon dioxide in figure 2. The similarly treated low-protein seedlings of the same strain are shown in figures 5 and 6.

Extra nitrogen supplied in the form of nitrates had a somewhat different influence on growth from that of additional carbon supplied as carbon dioxide. The high-protein Marquis wheat seedlings of experiment 1 (table 1) produced 12.5 grams of shoots and 9.9 grams of roots when grown without nitrates and 16.4 grams of shoots and 11.5 grams of roots when nitrates were furnished. The low-protein seedlings of the same strain produced 8.9 grams of shoots and 9.6 grams of roots when grown without nitrates and 17 grams of shoots and 12.7 grams of roots with nitrates supplied. Table 6



gives the percentage gain or loss over the checks due to the addition of nitrates. In columns 3 and 4 are shown the percentage differences in green weights of seedlings receiving carbon dioxide and nitrates in comparison to similar seedlings grown without nitrates. Shoots of low-protein seedlings of the two experiments gained 75, 90, and 79 percent and the roots 13, 31, and 18 percent, respectively. Seedlings of the high-protein strains did not benefit as much as those of the low-protein strains from the utilization of nitrates. Shoots of different lots of high-protein seedlings gained 45, 56, and 32 percent and the roots 19, 26, and 16 percent, respectively. Seedlings receiving nitrates had longer shoots but tended to have shorter roots than those not given nitrates. The most outstanding feature in the response of the carbon-dioxide-treated seedlings to nitrates is that the growth of shoots is favored much more than that of roots and the increase due to nitrates in the growth of shoots of low-protein seedlings is much greater than that of high-protein seedlings. The differences in appearance of the carbon-dioxide-treated seedlings grown with and without nitrates may be observed from the illustrations in Plate LXIX. Figures 2 and 4 show the high-protein seedlings and figures 6 and 8 the low-protein seedlings.

The use of nitrates without additional carbon dioxide resulted in the production of longer shoots but shorter roots than when nitrates were not used. Although longer leaves were produced with nitrates than without, the green weights of shoots were not increased in all cases. Somewhat different results with the use of nitrates but no extra carbon dioxide were obtained in experiments 1 and 2. In experiment 1, the dry weights of the low-protein seedlings receiving nitrates but no carbon dioxide were less than those of corresponding seedlings not furnished nitrates, indicating that the presence of nitrates may have stimulated the respiratory processes with the resulting loss of more of the dry matter (3). Table 1, columns 6 and 7, gives the dry weights of shoots and roots of seedlings receiving and not receiving nitrates and grown under conditions which restricted the synthesis of additional carbon dioxide. In experiment 2 in which the light was slightly less intense an apparent stimulation of respiration due to the presence of nitrates was not observable.

#### SIZES OF SEEDLINGS IN RELATION TO KIND AND AMOUNT OF FOOD RESERVES

Although the low-protein seedlings grown without extra nitrogen were smaller than those of high-protein seedlings, per unit of reserve nitrogen the seedlings of low-protein grains yielded greater weights of both shoots and roots. This response may be related to the greater amounts of reserve carbohydrates in proportion to nitrogen in the low-protein grains. The flinty grains of the high-protein Marquis wheat from Davis, California, which were used in experiment 2 contained 49 milligrams of nitrogen and the starchy low-protein grains from the same location and the same strain of wheat contained 32 milligrams of nitrogen. Although the starch or total

carbon content of these grains has not been determined quantitatively, microchemical examination indicated that the low-protein grains had a higher starch content. Per unit of reserve nitrogen, the low-protein starchy wheats contained much more starch.

# NITROGEN CONTENT OF TISSUES OF SEEDLINGS

Analyses of the nitrogen content of the roots and shoots of each set of seedlings were made and the results are given in tables 3 and 4. The same

TABLE 3. *Effect of CO<sub>2</sub> in the Atmosphere Upon the Capacity of Shoots and Roots to Utilize Nitrogen. April 24-May 6, 1926*

Variety of Wheat	Total Nitrogen in Seed %	CO <sub>2</sub> in Air Entering Chamber	Total Nitrogen from 88 Seedlings		Percentage of Nitrogen in Dry Weight		Percentage of the Total Nitrogen in:	
			Shoots mgs.	Roots mgs.	Shoots	Roots	Shoots	Roots
Without Nitrates								
Marquis * . . . . .	2.37	0.4%	47.3	18.3	3.14	2.16	72.1	27.9
Marquis * . . . . .	2.37	none	51.1	11.5	3.11	2.16		
Marquis † . . . . .	1.45	0.4%	28.9	13.2	3.96	3.02	81.6	18.4
Marquis † . . . . .	1.45	none	29.8	11.1	3.97	—		
Little Club † . . . . .	1.52	0.4%	28.5	13.8	1.90	1.50	68.6	31.4
Little Club † . . . . .	1.52	none	29.1	11.7	2.02	1.39		
					3.06	1.79	72.8	27.2
					2.81	1.65		
					2.11	1.55	67.3	32.7
					1.94	1.46		
					2.54	1.76	71.3	28.7
					2.31	1.73		
With Nitrates								
Marquis * . . . . .	2.37	0.4%	78.4	21.5	4.52	2.43	78.5	21.5
Marquis * . . . . .	2.37	none	65.3	16.4	4.48	2.55		
Marquis † . . . . .	1.45	0.4%	67.8	23.3	5.49	3.08	79.9	20.1
Marquis † . . . . .	1.45	none	46.4	10.2	5.55	3.14		
Little Club † . . . . .	1.52	0.4%	69.0	20.5	3.61	2.42	74.4	25.6
Little Club † . . . . .	1.52	none	45.2	9.2	3.53	2.25		
					5.02	1.90	81.9	18.1
					4.87	1.91		
					3.80	2.18	77.1	22.9
					3.69	1.99		
					5.45	1.94	83.1	16.9
					5.37	1.97		

\* Dickinson, N. D.

† Davis, Calif.

tables show the percentage of the total nitrogen of the plant found in the shoots and roots under the different environmental conditions. A higher percentage of nitrogen and also a greater amount of the total nitrogen of the

plant was found in the shoots of plants grown without additional carbon dioxide than in those to which carbon dioxide was given. The high-protein Marquis wheat seedlings of experiment 2 had 3 percent of nitrogen in the shoots and 2.1 percent in the roots with carbon dioxide supplied, and 5.1

TABLE 4. *Effect of CO<sub>2</sub> in the Atmosphere Upon the Capacity of Shoots and Roots to Utilize Nitrogen. May 10-22, 1926*

Seeds of Marquis Wheat Raised at:	Total Nitrogen in Seed %	CO <sub>2</sub> in Air Entering Chamber	Total Nitrogen from 75 Seedlings		Percentage of Nitrogen in Dry Weight		Percentage of the Total Nitrogen in:	
			Shoots mgs.	Roots mgs.	Shoots	Roots	Shoots	Roots
Without Nitrates								
Dickinson, N. D. . . . .	2.37	0.4%	40.0	14.0	3.09 3.04	2.07 2.06	74.1	25.9
Dickinson, N. D. . . . .	2.37	none	42.3	7.8	5.08 5.11	2.36 2.32	84.4	15.6
Davis, Calif. . . . .	2.11	0.4%	36.8	12.6	3.00 3.03	2.00 1.98	74.5	25.5
Davis, Calif. . . . .	2.11	none	40.8	7.7	4.04 4.01	1.82 1.79	84.1	15.9
Davis, Calif. . . . .	1.45	0.4%	26.1	11.4	2.16 2.21	1.58 1.58	69.5	30.5
Davis, Calif. . . . .	1.45	none	25.6	8.0	3.28 3.27	1.59 1.53	76.2	23.8
With Nitrates								
Dickinson, N. D. . . . .	2.37	0.4%	53.7	17.6	4.07 4.09	3.28 3.61	75.3	24.7
Dickinson, N. D. . . . .	2.37	none	64.6	12.7	5.94 5.84	3.29 3.15	83.5	16.5
Davis, Calif. . . . .	2.11	0.4%	69.5	20.2	4.02 4.11	2.68 2.70	77.5	22.5
Davis, Calif. . . . .	2.11	none	59.3	12.5	5.45 5.59	2.81 2.70	82.5	17.5
Davis, Calif. . . . .	1.45	0.4%	56.1	19.2	3.59 3.62	2.74 2.71	74.5	25.5
Davis, Calif. . . . .	1.45	none	49.8	10.5	5.21 5.18	2.62 2.83	82.6	17.4

percent in the shoots and 2.3 percent in the roots with no additional carbon dioxide. The nitrated seedlings had a higher nitrogen content than those not supplied with nitrates. The high-protein Marquis seedlings given nitrates had 4.1 percent nitrogen in the shoots and 3.4 percent in the roots with carbon dioxide furnished, and 5.9 percent in the shoots and 3.2 percent in the roots with no additional carbon dioxide supplied.

The seedlings of high-protein strains had greater differences in the distribution of the nitrogen in the plant as affected by carbon dioxide than those of low-protein strains. In experiment 2, the results of which are given

in table 4, the high-protein Marquis wheat seedlings grown from the Davis, California, wheat had 74.5 percent of the total nitrogen in the shoots and 25.5 percent in the roots when grown with carbon dioxide in the atmosphere and

TABLE 5. *Percentage Difference in Green Weight With Carbon Dioxid Added Over Paired Check Without It*

Class of Seeds	Date of Experiment	Nitrates Added		Nitrates not Added	
		Shoots	Roots	Shoots	Roots
High-protein.....	April 24-May 6	+ 41.2	+ 64.7	+ 3.3	+ 45.1
	May 10-22	+ 18.8	+ 63.5	+ 23.1	+ 76.5
	May 10-22	+ 25.5	+ 52.0	+ 5.1	+ 36.2
Low-protein.....	April 24-May 6	+ 102.8	+ 80.0	- 1.6	+ 29.9
	April 24-May 6	+ 100.2	+ 70.9	- 11.0	+ 28.3
	May 10-22	+ 23.7	+ 72.7	+ 6.9	+ 47.8

84 percent in the shoots and 16 percent in the roots when grown without carbon dioxide. The low-protein Marquis wheat seedlings of the same strain had 69.5 percent of the total nitrogen in the shoots and 30.5 percent in the roots when grown with carbon dioxide, and 76 percent in the shoots and 24 percent in the roots when grown without carbon dioxide. A similar influence

TABLE 6. *Percentage Difference in Green Weight With Nitrates Added Over Paired Check With Nitrates not Added*

Class of Seeds	Date of Experiment	With Extra CO <sub>2</sub>		Without Extra CO <sub>2</sub>	
		Shoots	Roots	Shoots	Roots
High-protein.....	April 24-May 6	+ 31.6	+ 16.0	- 3.6	+ 2.2
	May 10-22	+ 45.4	+ 18.6	+ 50.7	+ 28.2
	May 10-22	+ 56.4	+ 25.8	+ 30.9	+ 12.8
Low-protein.....	April 24-May 6	+ 90.5	+ 31.4	- 7.6	- 5.1
	April 24-May 6	+ 79.1	+ 17.9	- 20.4	- 11.4
	May 10-22	+ 74.6	+ 13.1	+ 50.9	- 3.1

of carbon dioxide on the distribution of nitrogen in the seedlings was also found in the seedlings which received nitrates. The results given in tables 3 and 4 also show the effect of composition of the seed upon the allocation of nitrogen in the seedling. Unnitrated seedlings grown from the low-protein, high-carbohydrate seeds have relatively more of their reserve nitrogen in the roots and less in the shoots than seedlings grown from the higher protein seeds.

#### GREENNESS OF LEAVES

Definitely noticeable differences in greenness of leaves developed under the various conditions for growth. For any given set of external conditions the seedling leaves of high-protein strains were greener than those of low-protein strains. It was also true that the higher the protein content of the wheat grains the greener the seedling leaves. For example, the leaves of the

very low-protein Marquis wheat seedlings grown from seed produced at Davis, California were less green than those of seedlings grown from the high-protein grains selected from the same sample of a relatively pure line wheat. The greenness of the leaves was also affected by the external environment. The higher the nitrogen in relation to available carbohydrates the greener the leaves. The following series indicates in decreasing order the greenness of leaves under different external conditions of carbon and nitrogen supply:



Unnitrated seedlings not receiving carbon dioxide had greener and also longer leaves than those that received carbon dioxide. The nitrated seedlings which were not given carbon dioxide also had greener leaves than those of seedlings supplied with carbon dioxide. The same differences in greenness of leaves under the varying conditions of carbohydrate and nitrogen supply were found in experiments 1 and 2. Seedlings of Illinois low-protein corn described in the previous paper, when grown without extra nitrogen were greener and longer, and there tended to be more visible leaves produced per plant if the seedlings were grown without carbon dioxide than if it were furnished.

#### SUMMARY

This investigation is along the same line as previous studies regarding the influence of the carbohydrate and nitrogenous storage reserves in the seed upon the subsequent development of the seedling and especially as to the proportion of shoots and roots produced. Wheat seeds of high and low protein content were grown in pulverized quartz under four sets of external conditions:

- (1) Without additional carbon dioxide and without nitrates;
- (2) With additional carbon dioxide and without nitrates;
- (3) Without additional carbon dioxide and with nitrates;
- (4) With additional carbon dioxide and with nitrates.

It was found that the weight of roots produced as compared with the weight of shoots depended not only upon the carbohydrate and nitrogenous reserves within the seeds but also upon the carbon dioxide content of the air. Seedlings of high-protein grains grown on their own nitrogen reserves had their total growth increased more by carbon dioxide than those of low-protein grains and the roots increased relatively more than the shoots. Both high- and low-protein seedlings grown without nitrates produced smaller tops and the leaves were less green when carbon dioxide was furnished than when it was withheld.

Whether or not a seedling responded to the addition of nitrates to the soil solution depended upon the relative amounts of carbohydrate and nitrogenous reserves in the seeds and the availability of carbon dioxide in the

external atmosphere. Seedlings of low-protein seeds grown in an atmosphere containing carbon dioxid had their total-growth increased with the utilization of nitrates more than that of high-protein seeds and the shoots were increased relatively more than the roots.

The most significant facts which appear are that addition of carbon dioxid causes an increased growth of roots, especially of high-protein seedlings, while the addition of nitrates, also, causes an increased growth of tops, and of low-protein seedlings more than of high-protein seedlings.

These investigations were conducted at Boyce Thompson Institute for Plant Research in 1926. I wish to thank the Cereals Division of the Bureau of Plant Industry, U. S. D. A., for supplying the wheat seeds.

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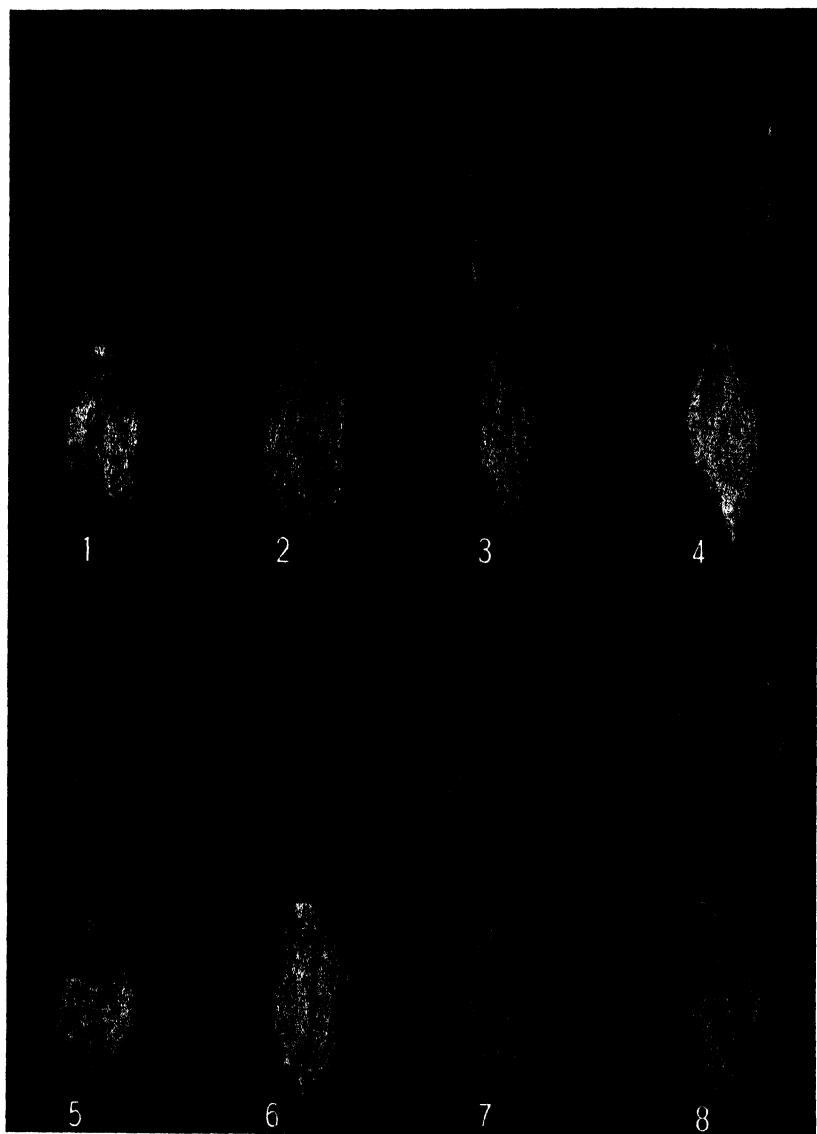
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#### EXPLANATION OF PLATE LXIX

- FIG. 1. High-protein Marquis wheat grown without carbon dioxid and without nitrates.
- FIG. 2. High-protein Marquis wheat grown with carbon dioxid and without nitrates.
- FIG. 3. High-protein Marquis wheat grown without carbon dioxid and with nitrates.
- FIG. 4. High-protein Marquis wheat grown with carbon dioxid and with nitrates.
- FIG. 5. Low-protein Marquis wheat grown without carbon dioxid and without nitrates.
- FIG. 6. Low-protein Marquis wheat grown with carbon dioxid and without nitrates.
- FIG. 7. Low-protein Marquis wheat grown without carbon dioxid and with nitrates.
- FIG. 8. Low-protein Marquis wheat grown with carbon dioxid and with nitrates.





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## PRIMARY DORMANCY, AFTER-RIPENING, AND THE DEVELOPMENT OF SECONDARY DORMANCY IN EMBRYOS OF *AMBROSIA TRIFIDA*

W. E. DAVIS

### INTRODUCTION

The fruits of *Ambrosia trifida* are dormant at maturity. The dormancy is in the embryo since germination does not take place even when the embryos are freed from all enveloping structures. An after-ripening process must precede germination, but even when the seeds are after-ripened their germination, especially at high temperatures, may be considerably delayed or prevented altogether due to the fruit and seed coats. Failing to germinate under such conditions the embryos develop a second dormant condition more pronounced than the initial or primary dormancy.

Since after-ripening, germination, the development of secondary dormancy, and possibly the primary dormancy of the embryo during its period of growth and maturity on the plant are more or less influenced by these membranes, a brief description of the embryo with its accompanying structures will be given at this place.

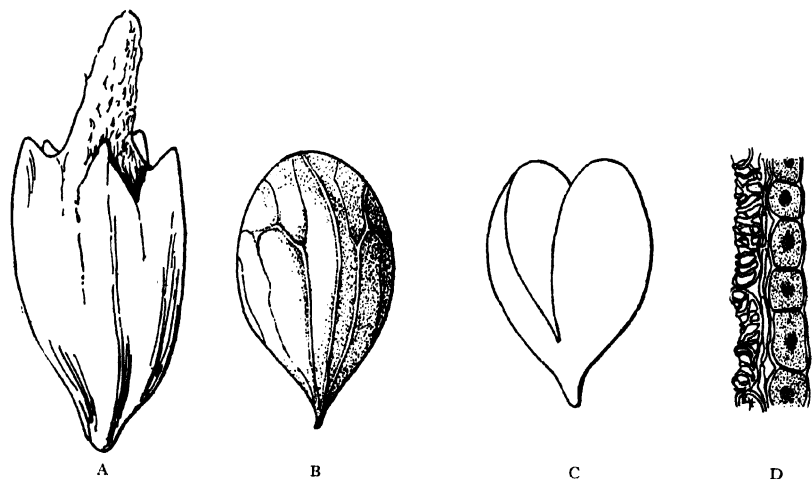
### STRUCTURE OF THE FRUITS

The seed-bearing structure is a fruit (text fig. 1 *A*), the outer part of which consists of a thick-walled involucre. Beneath the involucre is the ovary, the style of which protrudes through an opening at the top of the involucre. The walls of the ovary are distinct from the involucre. Within the ovary and readily separating from it is the seed (text fig. 1 *B*). The seed consists of the embryo encased by a delicate membrane usually two layers of cells in thickness, the outer of which consists of dead cells with striated thickenings in the walls together with fragments of disintegrated cell walls constituting the remnants of the integuments. The seed coat contains numerous vascular bundles (text fig. 1 *B*). The inner layer of cells in direct contact with the embryo is of nucellar origin. It is made up of living cells with moderately thick cell walls. The entire membrane (text fig. 1 *D*) may be separated from the embryo (text fig. 1 *C*) by pressing the soaked seed between the thumb and finger. With less soaking the

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outer layer of non-living cells separates readily from the living layer leaving the embryo with only the nucellar layer intact.

As these membranes (involucre, ovary wall, and seed coats) may each be removed without interfering with the structures beneath, their combined as well as their individual effect upon after-ripening, germination, and development of secondary dormancy in the embryo may be studied.



TEXT FIG. 1 A, the fruit; B, the seed; C, the embryo; D, section of the two-layered seed coat.

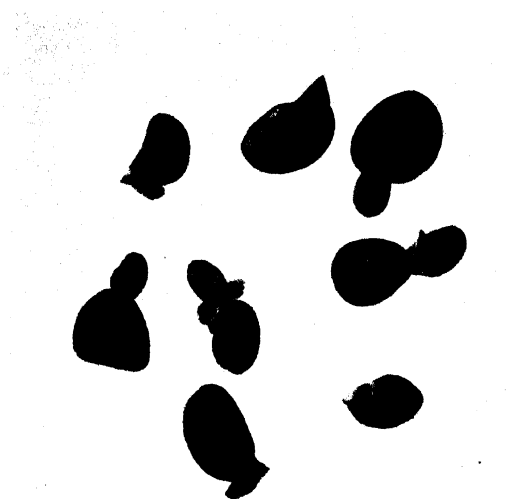
In this paper three structures are considered, as shown in text figure 1: (A) the fruit, (B) the seed obtained by the removal of involucre and ovary, and (C) the naked embryo. Dormant embryos in all cases have been after-ripened within the fruits or within the seed. Secondary dormancy of the embryo was usually induced within the fruit and seed coats, but also within the seed coats alone.

#### PRIMARY OR INITIAL DORMANCY

Although all embryos are dormant at maturity, the dormancy is not equally deep seated in all, since in dry storage it may disappear early from some and late or never completely from others. The degree of dormancy of different crops varies as indicated by results reported later.

Again the dormancy is not equally pronounced in all parts of the embryo itself but is especially characteristic of the hypocotyl as has been found true of most dormant embryos, such as *Crataegus* and others (1, 2, 3). When the naked dormant embryos are placed under germinating conditions, the hypocotyls fail to elongate and no roots are produced. The cotyledons, especially those in contact with the wet medium, frequently enlarge to

several times their original size and in the light become intensely green, while those not in direct contact with the medium remain colorless and of the original size. The plumule also frequently grows while the hypocotyl remains dormant. Text figure 2 shows these characteristic behaviors of the embryos upon moist cotton in petri dishes.



TEXT FIG. 2. Dormant embryos after a period in a germinator, showing growth of cotyledons and of some epicotyls in contact with moist substratum, and no growth of hypocotyls or of cotyledons not in contact with moist substratum.

It does not seem likely that these differences in degree of dormancy especially in embryos of the same crop can be attributed entirely to conditions that obtain during storage, for while the accessibility of oxygen due to differences in permeability of enveloping membranes may not have been the same to all embryos, the temperature and the moisture content during dry storage were the same.

The primary dormancy universally present in embryos at maturity has its origin during the development of the embryo while in contact with the mother plant. Since no attempt was made to store the fruits of different crops under the same conditions any differences in their relative dormancy after similar periods of storage may have been due in part to slight differences in temperature and moisture content during storage as well as to possible differences in permeability of enveloping membranes during development on the plant for the different years.

## AFTER-RIPENING IN DRY STORAGE

After-ripening or the disappearance of dormancy in embryos of fruits in dry storage, as indicated by germination, takes place slowly and very unequally in different embryos. In some, after-ripening may take place within a few months while in others the dormancy may still be present after one or more years. Excised embryos of fruits collected in 1918 when placed under germinating conditions, March 1919, gave five percent germination; in May, 36 percent, and in July, 40 percent. Excised embryos of fruits collected in 1919 gave 75 percent germination in October 1920. Embryos of fruits collected in 1924 gave 80 percent germination September 1926. Excised embryos of fruits collected in 1926 gave 40 percent germination August 1927. Embryos of fruits collected in 1927 gave no germination May 1928, while embryos of fruits collected in 1928 gave 40 percent germination July 1929. Naked embryos were used in all germination tests in order to determine whether the failure to germinate was due to the membranes surrounding the embryos or to a dormant condition of the embryos themselves. The germination tests were made at temperatures ranging between 27° and 29° C.

## AFTER-RIPENING IN LOW TEMPERATURE GERMINATOR

While after-ripening may gradually go on in the embryos in dry storage, it may be brought about much more quickly and uniformly when the fruits or seeds are placed upon a wet medium such as moist cotton at a temperature of from 5° to 10° C. The optimum temperature for after-ripening at low temperature is near 5° C. and requires, for freshly harvested fruits, from 70 to 90 days. Embryos of fruits after-ripened in the cold germinated also with greater energy than embryos of fruits after-ripened in dry storage.

Whether the after-ripening takes place in dry storage or at low temperature the process is evidently the same, since fruits that have been in dry storage for some time do not require as long a period to after-ripen in the cold as do recently harvested fruits. Fruits taken from dry storage and placed upon moist cotton in petri dishes at low temperature in March required less than two months to after-ripen while still later in the season one month was frequently found sufficient for the complete after-ripening of all fruits. Embryos of fruits that had been after-ripened in dry storage and subsequently given an additional period in the cold, germinated with greater energy than those taken directly from dry storage. Many of the intact fruits after-ripened at low temperatures, germinated overnight, and the hypocotyls of naked embryos often attained a length of from one to two centimeters within 24 hours after they had been removed from the fruits and placed under germinating conditions.

RELATION BETWEEN TEMPERATURE AND MOISTURE CONTENT  
IN AFTER-RIPENING

Embryos of fruits kept on moist cotton in petri dishes at a temperature varying from 23 to 25 degrees from November until July gave no germination at any time during that period. When the fruits were removed from these temperatures and placed in the cold, they required fully as long a period in the cold for the embryos to after-ripen as those of recently harvested fruits. The fruits that had been in a high temperature germinator since harvest were placed in a cold germinator July 7 and on September 26, when a portion of the fruits was removed from the cold, the naked embryos gave 70 percent germination. The remaining fruits were left in the cold until November when all embryos were found fully after-ripened, giving 100 percent germination within forty-eight hours. The after-ripening of the embryos in these fruits did not take place as uniformly as that of recently harvested fruits when placed in the cold germinator.

Fruits of ragweed were planted in pots in a greenhouse October 24, 1927. No germination had occurred up to May 5 when the fruits were removed from the soil. All membranes were then removed from around the embryos and the naked embryos were placed upon moist cotton at 28° C. to germinate. None of the embryos germinated. There had been no perceptible after-ripening of the embryos during the 194 days the fruits had remained in the soil at the temperature of the greenhouse.

In a previous year fruits planted in two pots *A* and *B* in the greenhouse October 30 gave 18 and 28 percent germination, respectively, by March 7. This difference in response to soil conditions may possibly be accounted for in that the embryos of the 1927 crop were more dormant than those of the previous year, or to a condition of the embryos when the fruits were planted, or even to the temperature of the greenhouse.

While there is an optimum temperature (5° C.) for after-ripening in the cold when the fruits or seeds are in a saturated condition, there appears also to be a close relation between the temperature and moisture content during after-ripening in dry storage. Excised embryos of fruits harvested and placed over concentrated sulfuric acid in November 1926 gave no germination at any time up to August of the following year.

In April 1927 after embryos of fruits in storage had begun to after-ripen, some of these air-dried fruits were placed in a closed vessel and transferred to a refrigerator at about 10° C. The embryos of these fruits after three months did not germinate as well as the embryos of the fruits left in storage in the laboratory. Embryos of fruits stored at the same time and at the same temperature in a container in which was placed a vial containing a few drops of water in order to raise slightly the moisture content of the embryos within the fruits, after-ripened more rapidly than the embryos of air-dried fruits stored in the laboratory.

The percentage germination of naked embryos of fruits subjected to the various treatments were: fruits stored over sulfuric acid, 0 percent; fruits stored in laboratory, 40 percent; fruits stored in closed vessel in refrigerator, 25 percent; fruits stored in refrigerator with moisture content slightly above that of air-dried fruits in the laboratory, 52 percent.

There is evidently not only a minimum moisture content below which after-ripening may not take place at all or only very slowly, but there is also a close relation between the moisture content of the embryo and the temperature employed. At low temperatures after-ripening takes place most rapidly when the water content is high or when the embryos are fully saturated. At high temperatures the after-ripening of embryos in air-dried fruits takes place slowly, while in fully saturated fruits it does not take place at all. At both low temperatures and high temperatures, the moisture content of embryos determines whether after-ripening will take place and also something of the rate at which it will take place. The process of after-ripening under any conditions requires a low respiratory intensity. At high temperatures and high moisture content the processes involved in after-ripening are apparently counteracted by those involved in respiration.

#### THE NECESSITY OF OXYGEN FOR AFTER-RIPENING

Whatever the changes may be that take place in after-ripening, a supply of oxygen to the embryo seems necessary to initiate and complete the changes. Air-dried fruits, the soaked embryos of which gave about 40 percent germination, were sealed in jars from which the oxygen was absorbed by potassium pyrogallate. Along with these as controls, fruits were sealed in other jars from which the oxygen was not absorbed. After one month at low temperature, the embryos of fruits taken from jars without oxygen gave no greater percentage germination than the embryos of similar fruits taken from dry storage at the time the experiment was started, while the embryos of fruits taken from jars with normal oxygen pressure at the beginning of the experiment, gave a much higher percentage germination.

In order to test further the oxygen requirement for after-ripening, petri dishes were prepared with layers of moist cotton. A solution of agar was poured over this layer of cotton and when sufficiently cooled fruits and seeds were arranged upon the agar.

A thin coating of agar was then spread over the fruits and seeds in the several dishes, after which they were placed at low temperature. Along with these were other dishes containing fruits upon moist cotton only. The seeds with a very thin coating of agar after-ripened almost as readily as the fruits upon the moist cotton alone, while the fruits covered with a thicker layer of agar after-ripened much more slowly. For example, the naked embryos of fruits covered with a thicker layer of agar after three months at low temperature gave only 70 percent germination while the embryos of fruits upon moist cotton gave 100 percent germination.

## CHANGES IN ACIDITY AND CATALASE ACTIVITY DURING AFTER-RIPENING

In a study of the after-ripening of seeds of *Crataegus*, Eckerson (3) found an increase both in acidity and in catalase activity.

During the after-ripening of embryos in fruits of *Ambrosia* at low temperature there was also a slight increase in acidity, probably due to hydrolysis of oils, together with a considerable rise in catalase, but it is doubtful whether either has any special significance in the process of after-ripening. Dormant embryos of fruits kept at high temperature in a saturated condition three months had an acidity slightly higher than that of air-dried seeds. Whether at low temperature or high temperature, the rise in acidity appears to be simply the result of metabolism in the embryo.

The rise in catalase, while an excellent index of after-ripening in the cold, seems also to have no special significance in the process of after-ripening, being a result and in no sense causal. Embryos of fruits in which there was a large percentage of embryos after-ripened in dry storage possessed no higher catalase content than the original dormant embryos. During the period of after-ripening in the cold there was always a considerable rise in the catalase content of embryos over that of air-dried ones. The average catalase content per embryo based upon a large number of both air-dried and after-ripened medium-sized embryos is indicated by the following data: the average cc. of  $O_2$  released per dry intact seed was 7.2 and for after-ripened seed 11.6.

The catalase content of individual air-dried seeds of approximately the same weight may differ, as shown in table 1. This is also true of seeds after-ripened in the cold, as shown in table 2.

TABLE 1. *Catalase Content of Individual Embryos from Air-dried Fruits*

Wt. in grams of Individual Embryos, Membranes Removed	Cc. of $O_2$ Released after 5 Minutes	Cc. of $O_2$ Released Based upon .1 gram
.0144.....	5.7	39.5
.0160.....	5.5	34.3
.0157.....	5.0	31.8
.0123.....	5.9	47.9
.0124.....	5.0	40.3
.0150.....	6.5	43.3
.0134.....	5.8	43.2
.0156.....	6.3	40.3
.0150.....	5.5	36.6
.0154.....	5.9	38.3
Ave. .01452.....	5.7	39.5

The embryos of fruits in table 2 were after-ripened in an ordinary refrigerator in which there was considerable fluctuation in temperature during the period of after-ripening. On this account the catalase content of the embryos may be higher than when after-ripened at a lower constant temperature.



TABLE 2. *Catalase Content of Individual Embryos Fully After-ripened in a Low-temperature Germinator. Taken from the Same Lot of Air-dried Fruits as Employed in Table 1*

Wet Wt. in grams of Individual After-ripened Embryos	Cc. of O <sub>2</sub> Released after 5 Minutes	Cc. of O <sub>2</sub> Released Based upon .1 gram
.0150.....	10.0	66.6
.0165.....	11.5	69.6
.0140.....	10.0	71.4
.0197.....	11.5	58.3
.0182.....	13.0	71.4
.0198.....	12.2	61.6
.0150.....	8.3	55.3
.0176.....	10.3	58.5
.0228.....	18.5	81.1
.0183.....	12.3	67.2
Ave. .01769.....	11.76	66.1

## GERMINATION OF AFTER-RIPENED FRUITS

When fruits of *Ambrosia trifida*, after-ripened in the cold, were placed upon a moist medium at a temperature between 25° and 30° C., usually a large percentage of fruits germinated within a few days, but there was always a number of fruits that did not germinate, not because the embryos within were not fully after-ripened, but because of restrictions imposed upon the embryos by membranes that surrounded them. When these membranes were removed, the naked embryos readily germinated. The percentage germination of after-ripened fruits depends upon the extent to which the after-ripening has been carried and upon the temperature at which they are placed to germinate. At temperatures around 20° C. the germination usually takes place more slowly than at temperatures near 30° C. but may extend over a longer period of time, and give a higher final percent. For example a batch of fruits was left at low temperature for three months after which they were placed to germinate, some at 30° C. and some at 22° C. After eight days 80 percent of those at 22° had germinated while only 27 percent of those at 30° had germinated. At another time two lots of after-ripened seeds of 40 each were placed at 27° and 20° C., respectively, and after seven days 52 percent of those at the higher temperature had germinated and only 35 percent of those at the lower temperature. A temperature of 30° C. is slightly above the optimum for germination of embryos inclosed within fruits.

When fruits after-ripened in the cold and especially fruits that have after-ripened in dry storage were subjected to alternating temperatures the percentage of germination was much greater than at either temperature used in the alternation, as is indicated in tables 3 and 4. In table 3 the fruits were removed from the cold as soon as the naked embryos were found capable of complete germination. In table 4 the fruits were taken from dry storage at which time about 40 to 50 percent of the naked embryos responded to germinating conditions.

TABLE 3. *The Effect of Alternating Temperatures upon Germination of Fruits After-ripened at Low Temperature*

Number Employed	Condition	Temperature (° C.)	Percentage Germination after 12 Days
50.....	Fruits	20	50
50.....	"	20-30	70
50.....	"	30	24
50.....	Excised embryos	30	100

TABLE 4. *The Effect of Alternating Temperatures upon the Germination of Fruits in Dry Storage, About 40 Percent of the Naked Embryos of Which Were Capable of Germinating*

Number Employed	Condition	Temperature (° C.)	Percentage Germination after 12 Days
50.....	Fruits	20	4
50.....	"	20-30	24
50.....	"	30	2

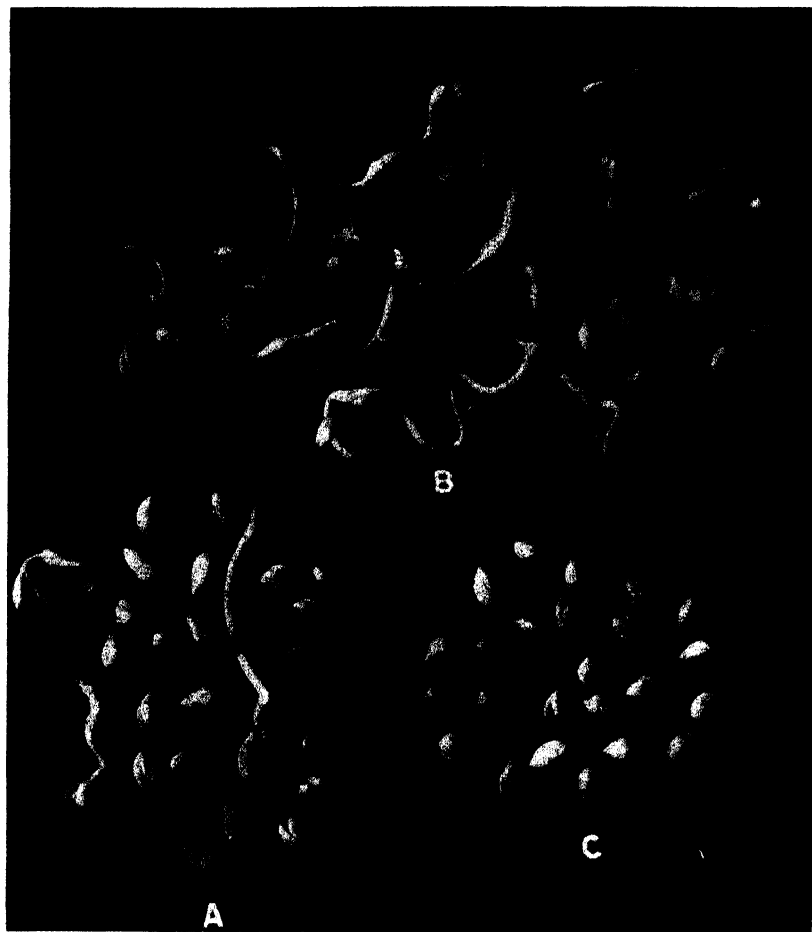
The germination of fruits evidently depends upon the degree of after-ripening, the temperature, and the oxygen available to the embryo within the fruits. The degree of after-ripening determines the capability of the embryo to respond to the conditions of germination. The oxygen supply is controlled by the membranes incasing the embryo and also by the temperature. At high temperatures an oxygen supply commensurate with the demands of the embryos cannot be maintained in all intact fruits. As a result of such limited oxygen intake embryos that are not thoroughly after-ripened or even when fully after-ripened as indicated by their ready germination when naked, are often prevented from germinating.

#### THE DEVELOPMENT OF SECONDARY DORMANCY IN EMBRYOS OF FRUITS AT HIGH TEMPERATURE

The after-ripened fruits or seeds of *Ambrosia trifida* that do not germinate at high temperatures do not die, but after a time revert to a dormant condition and must again be returned to the cold and go through another after-ripening process before germination can take place. It usually requires as long a period in the cold to overcome this induced or secondary dormancy in the embryo as is required to overcome the original or primary dormancy.

The time required for the after-ripened embryos within the fruit to revert to a state of dormancy in which even the naked embryos do not under any condition germinate, varied considerably. At one time fruits that required 92 days to after-ripen were removed from the cold and placed at a temperature around 28° C. to germinate. After 32 days the embryos of fruits that had not germinated were found to have reverted to the dormant

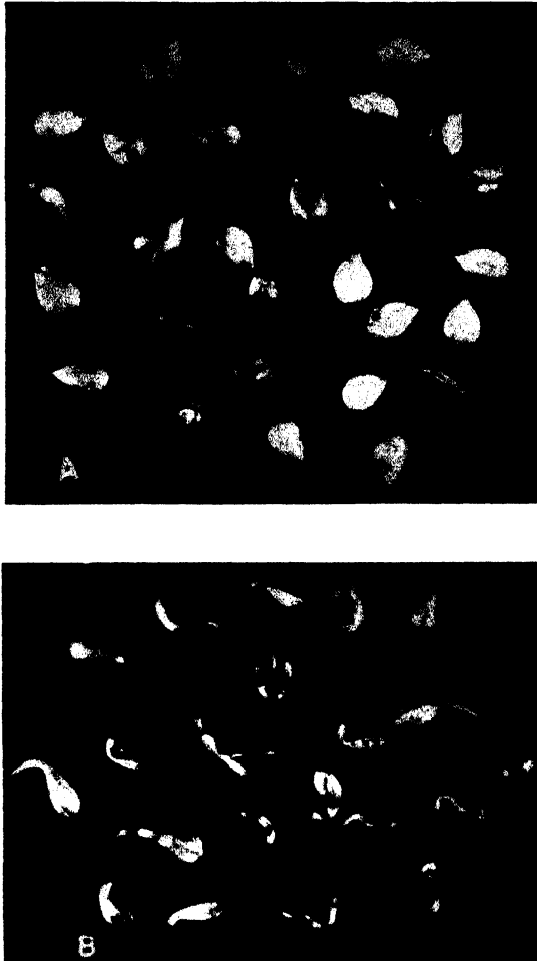
condition. These dormant fruits were again returned to the cold where they were allowed to remain 100 days, when the naked embryos were again found capable of germinating.



TEXT FIG. 3. Germination of embryos taken from fruits that had been stored for three months as follows: *A*, dry; *B*, in a germinator in a refrigerator; and *C*, in a germinator at 27°-30° C.

In July 1927 the embryos of fruits after-ripened in the cold, when placed at 29° C. for one month still gave 30 percent germination. In June 1929 both fruits and seeds, between 30 and 40 percent of the naked embryos of which would germinate, were placed upon moist cotton in petri dishes between 27° and 30° C. After 30 days, naked embryos from both fruits

and seeds were found incapable of germinating. Embryos sufficiently after-ripened in dry storage to germinate are rendered dormant more quickly than embryos of fruits after-ripened in the cold. Fruits taken from the same lot as above usually after-ripened in the cold in about a month. At



TEXT FIG. 4. *A*, embryos from seeds that have been through primary and secondary dormancy and two after-ripening periods. *B*, similar seeds after an overnight period in a germinator, showing the vigor with which they germinate.

this stage of after-ripening in dry storage the embryos within the fruits or seeds may quickly be either after-ripened or rendered dormant, depending upon the temperature to which they are subjected.

In text figure 3 are shown the effects upon the embryos of the different treatments to which the fruits have been subjected. All fruits had been in dry storage until January when they were divided into three lots. One lot was left in dry storage. A second was placed upon moist cotton in a petri dish in the refrigerator, while the third lot was placed upon moist cotton in petri dishes at temperatures between 27° and 30° C. After three months the embryos in each lot were freed from the fruits and the embryos were placed under germination conditions. Text figure 3 *A* represents the embryos of fruits in dry storage after four days in the germinator; *B*, embryos of fruits in the cold after three days in the germinator; and *C*, embryos of fruits at high temperature after four days in the germinator. No further germination took place in *A* and *C* after they were photographed.

On October 27, 1924, fruits were placed upon moist cotton in petri dishes at 5° C. to after-ripen. On February 1 of the following year when the excised embryos of all fruits responded readily to germinating conditions, the fruits were removed from the cold and placed at a temperature of 27° C. They were left at this temperature until April 29 when the embryos of the fruits that had failed to germinate up to this time were again found to have entered into a dormant condition and were returned to the cold where they were not molested until August 28, when they were again transferred to high temperature. Text figure 4 *A* shows embryos removed from fruits when taken from the cold the second time. Text figure 4 *B* shows some of these after-ripened embryos after an overnight period in a germinator at high temperature. These fruits, including the periods of time at both high and low temperatures, had spent more than 300 days in a practically saturated condition and had during that time passed through two dormant and two after-ripening periods without apparent injury.

#### EFFECT OF ENVELOPING MEMBRANES UPON THE GASEOUS EXCHANGE IN EMBRYOS WITHIN FRUITS AND SEEDS

Since the embryos of after-ripened fruits germinated readily when the membranes described above had been removed and since the development of dormancy is dependent upon the presence of these same membranes, their effect upon the gaseous exchange or respiration of the embryos within the fruit or the seed will be considered.

The data in table 5 show the combined effect of all the membranes taken together upon the gaseous exchange of the embryos in the fruit; the effect of the nucellar membrane alone; and the gaseous exchange that takes place in the embryos when freed from all membranes. Twenty fruits, seeds, or embryos were used in each experiment. The fruits used and the fruits from which the seeds and embryos were obtained had been in dry storage in the laboratory. No special attempt was made to select fruits, seeds, or embryos of the same weight or size, although for the same

numbers of each the weights were fairly uniform. The respiration was determined by a closed type of respirometer described by Harrington and Crocker (4).

TABLE 5. *Respiration at 30° C. after 20 Hours*

Fruits			Seeds			Embryos		
Cc. O <sub>2</sub> Taken up	Cc. CO <sub>2</sub> Released	$\frac{\text{CO}_2}{\text{O}_2}$	Cc. O <sub>2</sub> Taken up	Cc. CO <sub>2</sub> Released	$\frac{\text{CO}_2}{\text{O}_2}$	Cc. O <sub>2</sub> Taken up	Cc. CO <sub>2</sub> Released	$\frac{\text{CO}_2}{\text{O}_2}$
1.55 .....	1.46	.94	1.65	1.16	.70	3.73	2.55	.68
1.10 .....	1.09	.99	1.69	1.21	.71	4.12	2.83	.68
1.32 .....	1.24	.94	1.52	1.02	.67	3.91	2.73	.69
1.17 .....	1.08	.92	1.69	1.16	.68	3.98	2.80	.70
1.46 .....	1.28	.87	1.68	1.15	.68	4.02	2.86	.71
1.08 .....	.94	.87	1.83	1.29	.70	3.31	2.22	.67
1.43 .....	1.34	.93	2.01	1.36	.67	3.92	2.70	.69
1.24 .....	1.08	.87	1.81	1.22	.67	3.28	2.16	.65
			1.59	1.11	.69			
Ave. 1.29....	1.19	.92	1.72	1.19	.69	3.78	2.61	.68

TABLE 6. *Respiration of Seeds and Embryos at 15° C. after 20 Hrs.*

Seeds			Embryos		
O <sub>2</sub>	CO <sub>2</sub>	$\frac{\text{CO}_2}{\text{O}_2}$	O <sub>2</sub>	CO <sub>2</sub>	$\frac{\text{CO}_2}{\text{O}_2}$
1.28 .....	.75	.58	1.88	1.16	.61
.93 .....	.56	.60	1.82	1.14	.62
.77 .....	.50	.60	2.05	1.25	.61
1.18 .....	.69	.58	1.77	1.09	.61
.96 .....	.58	.60	1.92	1.20	.63
1.05 .....	.59	.56	1.94	1.19	.61
1.10 .....	.74	.67	1.59	.99	.62
Ave. 1.08 .....	.63	.60	1.85	1.15	.62

It will be observed that when the membranes are removed, the gaseous exchange between the embryo and the surrounding air is increased. The nucellar membrane is more effective in reducing the volume of oxygen taken up and carbon dioxide given off by the embryo, than that of the involucre and ovary wall combined.

The nucellar membrane of the seed in text figure 1 D at 30° C. has reduced the gaseous exchange of the seed to less than one-half that of the naked embryo. It does not seem to affect greatly the respiratory ratio since it is about the same for seeds with the nucellar membrane intact as for the naked embryos.

In the fruits the respiratory ratio is higher than that obtained for either seeds or embryos. The ratio in the fruits also lacks uniformity. This lack of uniformity and possibly the high respiratory ratio may be due

to different degrees of saturation of the involucral and ovary walls or to variations in the films of water between the membranes.

At 15° C. as at 30° C. a great reduction has taken place in the volumes of the gases taken up and given off by seeds over that of naked embryos due to the presence of the nucellar membrane. The respiratory ratio is also lower than that at the higher temperature, but its value, as for the higher temperature, is practically the same for both seeds and naked embryos.

#### OXYGEN NECESSARY FOR INDUCING DORMANCY

While a restriction of the oxygen pressure by the membranes of seeds was found necessary for the development of dormancy, yet it does not develop in the absence of oxygen, as was found true for after-ripening. After-ripened fruits and seeds, when covered with a coating of agar by which the oxygen supply was greatly reduced, became dormant much more slowly than those not so treated. The optimum oxygen pressure in the development of dormancy, while not determined, may possibly be that pressure which at a given temperature falls just short of causing germination.

#### THE RELATION BETWEEN CATALASE ACTIVITY AND RESPIRATION

The catalase activity of embryos held in germinators at different temperatures with and without the various membranes, parallels closely the respiration intensity under similar conditions. The highest catalase activity is found in the naked embryos at all temperatures, and the least activity in the fruits where the oxygen supply is least. Fruits, seeds and embryos were placed upon moist cotton in petri dishes and kept at 25° C. for three days, after which the catalase content was determined for each.

In table 8 fruits, seeds, and embryos were prepared as in table 7 and placed at a temperature of 15° C. for 20 days. At the end of this period all membranes were removed from the fruits and seeds and the catalase content was determined for the three sets of embryos.

TABLE 7. *Catalase Activity after 3 Days in a Germinator at 25° C.*

Number Used	Condition During Treatment	Cc. O <sub>2</sub> Released after 10 Minutes	Average cc. O <sub>2</sub> per Embryo Released
3	Fruits	29	9.7
3	"	33	
3	"	25	
3	Seeds	43	14.2
3	"	42.5	
3	Embryos	70	
3	"	80	25.4
3	"	79	

In table 9 the treatment was similar to that in tables 7 and 8 except for temperature and time. They were kept at 30° C. for 10 days after which the catalase was determined on the embryos of each.

TABLE 8. *Catalase Activity after 20 Days in a Germinator at 15° C.*

Number Used	Condition During Treatment	Wt. in grams (wet wt.)	O <sub>2</sub> Released after 10 Minutes	Average cc. O <sub>2</sub> per Embryo Released
2	Fruit	.093	52.3	25.8
2	"	.085	51.2	
2	"	.085	51.5	
2	"	.078	48.8	
2	"	.088	54.5	
2	Seeds	.090	59.5	28.5
2	"	.087	62.8	
2	"	.090	62.0	
2	"	.080	46.3	
2	"	.090	55.0	
2	Embryos	.090	106.0	49.5
2	"	.100	106.5	
2	"	.097	91.5	
2	"	.092	92.5	

TABLE 9. *Catalase Activity after 10 Days at 30° C.*

Number Used	Condition During Treatment	Wt. in grams (wet wt.)	O <sub>2</sub> Released after 10 Minutes	Average cc. O <sub>2</sub> Released per Embryo
2	Fruit	.080	30.2	14.3
2	"	.092	32.0	
2	"	.087	29.7	
2	"	.090	25.6	
2	"	.078	25.5	
2	Seeds	.087	28.5	12.1
2	"	.090	20.2	
2	"	.085	19.2	
2	"	.090	25.7	
2	"	.090	27.5	
2	Embryos	.092	51.5	26.2
2	"	.093	47.5	
2	"	.094	54.5	
2	"	.088	54.0	
2	"	.088	54.5	

At 30° C. the catalase of the embryo treated in the fruit is slightly higher than for seeds where the nucellar membrane is present. This was found true elsewhere when seeds were run at 30° C. but never true at lower temperatures.

The respiration increases as the temperature increases but the catalase of the embryos treated within the fruit or seed above a certain temperature tends to decrease. Both seem to depend upon the oxygen supply since a removal of the membranes tends to increase at about the same ratio both the respiration and the catalase content of the naked embryos over that of embryos with membranes intact. Overholser (8) found a marked increase in catalase activity in Vicar pears at 15° C., but at temperatures above that a decrease in catalase activity. Lantz (7) reported an accumulation of catalase in corn germinating at 10° C., but at higher temperatures a decrease. Somewhere between 15° and 20° C. for dormant seeds or fruits



of ragweed the catalase content for some time at least is fairly constant. Below that temperature as the embryos after-ripen there is an increase in catalase, while above it the catalase decreases. In table 10 is shown the catalase activity of fruits after 10 days at various temperatures.

TABLE 10. *Catalase Activity of Fruits after 10 Days at Various Temperatures*

Number of Fruits	Wt. of 10 Embryos in grams (wet wt.)	Temperature (° C.)	Average cc. of O <sub>2</sub> per Embryo Liberated after 10 Minutes
10	.350	0	19.3
10	.355	5	20.7
10	.352	10	21.2
10	.380	15	19.5
10	.365	20	15.9
10	.362	27	13.8
10	.365 (air dry)		18

### THE CAUSES OF DORMANCY

Since dormancy in embryos of *Ambrosia trifida* develops only at rather high temperatures, its cause seems to be due to the restricted respiration at these temperatures imposed upon the embryo by the membranes that envelop it. The nucellar membrane plays the principal rôle here. This membrane, when the seed is in a germinator at 30° C., reduces the gaseous exchange of dormant embryos to less than one half of that of naked embryos. During the period in which the after-ripened embryos become dormant there is a decrease in the intensity of the respiration in the embryos inclosed within the fruit and seed coats. The catalase content of the embryo also decreases during this time, indicating a relation between catalase activity and the respiration. Although the catalase may have no function in either after-ripening or dormancy since in either case it seems to be a result rather than a cause, yet a comparison of its activity determined from time to time upon imbibed seed at different temperatures serves as a fairly accurate indicator as to what processes are in the ascendancy in the embryo. Its rise or fall, accompanied as it is by a rise or fall in respiratory intensity, also indicates closely the temperatures at which the oxygen supply to the embryo is sufficient or deficient.

Just what takes place in the development of dormancy in seeds of *Ambrosia*, one cannot say. Kidd and West (5) were able to produce dormancy in seeds of white mustard by means of carbon dioxid, but the dormant condition tended to disappear by a removal of seed coats or a redrying of the seed. In the embryos of *Ambrosia* the induced dormancy is so deep-seated that neither redrying nor the complete removal of all membranes from the embryo will bring about germination. Partial intramolecular respiration induced at high temperatures by a restriction of the oxygen supply, together with an increase in the pressure of carbon dioxid over

that of oxygen, may bring about a sort of asphyxiation which in time may render the embryo incapable of germinating under the most favorable conditions. A slight initial rise in both the respiration and the catalase when the seeds and fruits are first placed at high temperatures followed later by a depression in each with a rising respiratory ratio indicate a partial intramolecular respiration.

On the other hand, it is possible that the restricted respiration which is necessary to produce dormancy depletes certain necessary nutrient substances or develops inhibiting substances. Since the recovery from pronounced dormancy of the embryo is brought about by subjecting the imbibed fruits or seeds to a prolonged period in a cold germinator, development of after-ripening may involve, respectively, reduction and oxidation processes in the embryo. Since oily seeds like *Ambrosia* with dormant embryos have at low temperatures a very low respiratory ratio, much more oxygen being taken up than carbon dioxide given off, some of this oxygen may possibly be fixed in the formation of more or less unstable compounds which become readily available to the embryo in germination. The rapidity and the energy which the fully after-ripened embryos exhibit in germination indicate this. At high temperatures, on the other hand, when germination is prevented by the restriction of the oxygen supply to the embryo by the enveloping membranes, oxygen may perhaps be withdrawn from compounds stored in the embryo, reducing them to more stable compounds which are not available to the embryo. The two processes, after-ripening of dormant embryos and the development of dormancy in after-ripened embryos, are opposite in nature. After-ripening involves changes, whatever they may be, that are associated with a low respiratory process which in fully imbibed embryos is attained only at low temperatures. It involves as it were a rest and a recuperation after a period of activity at high temperatures, during which the embryo has been deprived of its ability to respond to the conditions of germination.

Even after-ripening in air-dried fruits requires a considerable period of time and apparently does not take place in the embryos of some fruits at all. The after-ripening of embryos within air-dried fruits is not dependent upon the removal of water alone. Even here there is evidently some readjustment in the embryo itself, which requires considerable time. In some embryos the dormancy is so pronounced that it cannot be overcome in dry storage. It has also been demonstrated that at least some water in the embryo is necessary to induce the changes involved in after-ripening since fruits placed in a closed vessel over sulfuric acid did not after-ripen.

#### DORMANCY IN EMBRYO OF SEEDS IN NATURE

Many seeds are dormant at maturity; especially is this true of seeds of the *Rosaceae*. Some seeds that are dormant at maturity after-ripen under dry storage conditions, but in many the dormancy is so deep-seated

that it can only be overcome by a prolonged period in a moist condition at low temperature. The dormancy in seeds of hawthorn apparently is not diminished in dry storage and the seeds finally pass from the dormant to the lifeless condition.

As to the cause or causes of dormancy in nature one can only surmise. There may be one cause or several, but judging from the conditions under which the seeds of *Ambrosia trifida* may be made to pass in the laboratory from a condition in which they germinate readily, to a condition in which no germination will take place even with the naked embryos, one may conclude that the same causes are operating in nature as in the laboratory. Seeds in which the embryos are dormant usually mature at the end of the season so that they have a rather long period of development at high temperatures. These seeds, as far as I am aware, at least when mature, also have membranes that restrict more or less the gaseous exchange in the seed. The restriction of the oxygen supply acting through a long period even at moderately high temperatures may bring about a state of dormancy in these embryos. In all cases where dormancy in the embryo is involved, whether produced in nature or in the laboratory, there is no doubt a very close relation between dormancy and restricted respiration.

#### SUMMARY

1. The embryos of fruits of *Ambrosia trifida* are dormant at maturity.
2. The embryos of the fruits after-ripen slowly in dry storage, but much more rapidly and completely in a saturated condition at low temperature ( $0^{\circ}$ – $10^{\circ}$  C.).
3. The time required for the after-ripening of freshly harvested fruits at the optimum low temperature ( $5^{\circ}$  C.) is about three months.
4. During the period at which the fruits are at low temperature there is a slight rise in the acidity together with a pronounced rise in the catalase content of the imbibed embryos over those of air-stored fruits.
5. If the embryos after-ripened in either dry storage or at low temperatures fail to germinate when placed at high temperatures, due to enveloping membranes that interfere with the gaseous exchange between the embryos and the external atmosphere, they revert to the dormant condition and must again be after-ripened before germination will take place.
6. The time required for the development of secondary dormancy in the after-ripened embryos is from 30 to 60 days.
7. The same embryos of fruits may be repeatedly made dormant and after-ripened by alternating periods at high temperature with sub-minimal oxygen supply and with periods at low temperature with adequate oxygen supply in water imbibed condition.
8. The cause of secondary dormancy in embryos of *Ambrosia trifida* appears to be due to restricted respiration at high temperatures.
9. The cause of restricted respiration is the low permeability of the membranes, which envelop the embryo, to oxygen.

10. The restriction of the gaseous exchange is due mainly to the influence of the nucellar membrane, consisting of a single layer of living cells immediately enveloping the embryo.

11. While the development of dormancy is associated with restricted or incomplete respiration, the presence of a supply of oxygen seems necessary to its development. Embryos of fruits embedded in agar in order to reduce further the oxygen supply to the embryos developed dormancy more slowly than embryos of fruits at the same temperature but free from agar.

12. During the period in which the embryos become dormant both the respiration and the catalase activity are reduced.

13. At the germination temperatures employed the catalase activity of fruits, of seeds, and of naked embryos bear about the same relation to one another as does the respiratory capacity of each of these to the other.

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## THE DEVELOPMENT OF DORMANCY IN SEEDS OF COCKLEBUR (*XANTHIUM*)

W. E. DAVIS

### INTRODUCTION

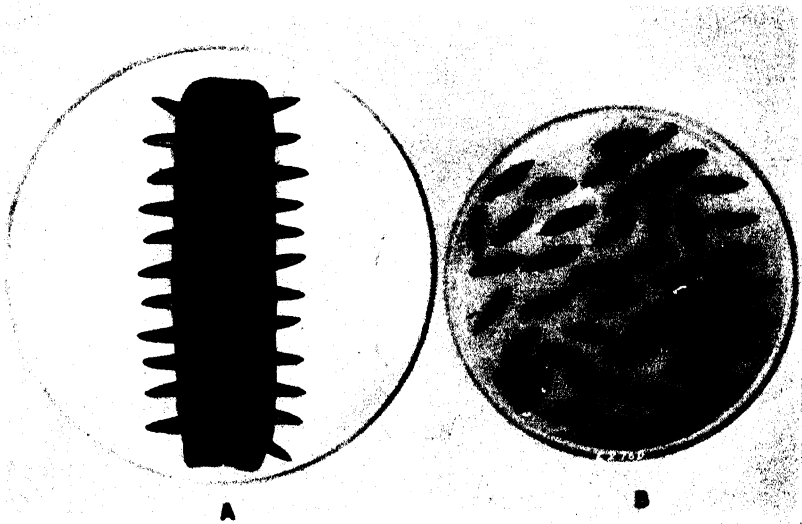
In a previous paper (2) it was shown that dormancy may be induced in the after-ripened embryos of ragweed (*Ambrosia trifida* L.) by means of high temperature germination in connection with restricted oxygen supply to the embryos due to the fruit and seed membranes that envelop them. These embryos, however, were dormant at maturity and the induced secondary dormancy was evidently merely a reversal of the essential changes through which the embryos had gone in the after-ripening process or the removal of the original or primary dormancy. The embryos of seeds of *Xanthium canadense* and *X. commune* have at no time during periods of dry storage of seeds in the burs exhibited any tendency to dormancy when placed under germinating conditions. No doubt this is also true of other species of *Xanthium*. Shull (4) compared the germination of embryos of seeds of *X. glabratum*, when quite green, with those of fully ripened seeds and of seeds one year old and was able to detect no perceptible after-ripening in passing from the unripe to the ripe and year-old conditions. The so-called dormancy or delay in the germination of these seeds at certain temperatures is due, as pointed out first by Crocker (1), to the restriction of the oxygen supply to the embryos by the seed coats for when the seed coats are removed and the naked embryos are placed under suitable conditions, germination usually takes place within 24 to 48 hours.

### DEVELOPMENT OF DORMANCY IN SEEDS OF *XANTHIUM* UNDER LABORATORY CONDITIONS

Since there is this restriction of the gaseous exchange between the embryos and the outside air by the seed coats, it was thought highly probable that a dormant condition might be induced in the embryos themselves provided germination could be prevented at temperatures necessary to produce dormancy. While the restriction of the oxygen supply by the seed coats in *Xanthium* seeds is normally considerable as shown by Crocker (1) and Shull (4), it is not sufficient to prevent germination at temperatures

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necessary to produce pronounced dormancy. In order to reduce the gaseous exchange to a point where germination would not take place even at high temperatures, the hypocotyl ends of soaked seeds were embedded in modeling clay so that about one-third of the length of the upper seeds and even less of the lower seeds of burs were exposed to view. The clay with the embedded seeds was placed upon very moist absorbent cotton in petri dishes so that the exposed parts of the seeds were in contact with the wet medium. By this means seeds could be placed at once at temperatures as high as 30° C. without great loss through germination. After a few weeks in this condition, the embryos of the seeds had become sufficiently dormant to be removed from the clay and placed upon wet cotton and returned to



TEXT FIG. 1. *A*, seeds in clay in a high temperature germinator to produce dormancy; *B*, imbedded in agar for the same purpose.

the same temperature to which they previously had been exposed while in the clay. A period of from eight to ten weeks in the clay was usually sufficient to prevent germination of the seeds when transferred from the clay to the moist cotton alone.

More recently agar has been employed in preventing germination at high temperatures and has been found even more effective than clay. A solution of three or four percent agar was prepared and poured over sterilized moist cotton in petri dishes. When the solution of agar had sufficiently cooled so as not to cause injury to the seeds, the sterilized seeds that had previously been soaked overnight in water were arranged upon the agar. An additional thin layer of agar was now spread over the seeds.

The upper seeds of burs required only a very thin layer of agar to prevent germination while the lower seeds required a considerably thicker coating. Since the seeds arranged in the agar were nowhere in contact with one another, any seed that showed signs of decay while at high temperature in the incubator was readily removed without disturbing others. It was found necessary to soak all seeds before embedding in either clay or agar since the soaking brought out all defects in seed coats due to injury in the removal of the seeds from burs. All seeds with defective coats either germinated or soon decayed. Text figure 1, *A* and *B*, shows seeds embedded in clay and in agar, respectively.

Whether the dormancy was produced by embedding the seeds in agar or clay, the naked embryos exhibited many of the characteristics of dormant and partially dormant embryos as previously pointed out for dormant naked embryos of hawthorn (*Crataegus mollis*) by Davis and Rose (3) and by Davis (2) for embryos of ragweed (*Ambrosia trifida*). There was often a slight elongation of the hypocotyl, and a tendency for the cotyledon in contact with the wet medium to enlarge and become green while others not in contact with it often failed to enlarge and remained colorless. There was also a tendency for the cotyledons by unequal growth of the upper and lower surfaces to cause the embryos to become inverted upon the medium leaving the hypocotyl pointing upward. In some embryos the plumules showed slight growth while the hypocotyls remained inert.

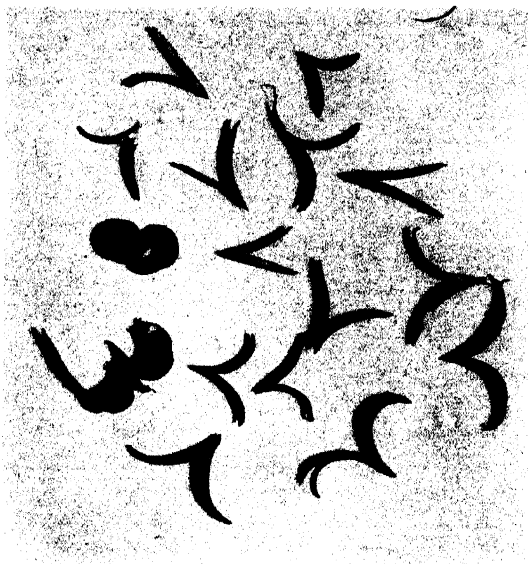
TABLE 1. *Results with Upper Seeds Held at a Temperature of About 28° C. for 140 Days, Including the Periods in Clay and on Moist Cotton*

Number of Embryos Germinated after 13 Days.....	1
"    "    "    "    "    16    "    .....	2
"    "    "    "    "    18    "    .....	5
"    "    "    "    "    19    "    .....	7
"    "    "    "    "    24    "    .....	9
"    "    "    "    "    30    "    .....	10

Table 1 shows the results of upper seeds of burs that were kept at a temperature of about 28° C. for 140 days, including both the period in the clay and the period the intact seeds were upon the moist cotton only. At the end of the period the seed coats were removed and the embryos were placed under germinating conditions. The number of seeds used was 11.

The remaining embryo had not germinated at the end of 30 days. The embryos of similar but untreated seeds which had been soaked 24 hours in water in order that the seed coats might be removed, gave 100 percent germination within 24 hours. The seed coats of untreated seeds as well as those of treated seeds were removed in all experiments involving germination in order to determine whether the dormancy was due to the seed coats, the permeability of which might have been altered during the treatment, or whether it was due to a condition induced in the embryos themselves.

Text figure 2 shows embryos of seeds that had been exposed to temperatures ranging from 27° to 30° C. for four months. During one-half of this time the hypocotyl ends of the seeds were embedded in clay while the remainder of that time the intact seeds were upon moist absorbent cotton



TEXT FIG. 2. Dormant embryos of cocklebur that have been in a high temperature germinator for 30 days.

only. The seed coats were then removed and the embryos returned to the incubator. The embryos were photographed 30 days later. Several of the embryos germinated during this time and were removed from the germinating chamber and so are not shown.

#### DEVELOPMENT OF DORMANCY IN EMBRYOS OF SEEDS OF XANTHIUM IN NATURE

Embryos of seeds collected at various times of the year from plants standing in fields at no time showed any tendency to dormancy. Since, however, a high moisture content has been shown to be an important factor in the development of dormancy in embryos one would not expect to find any marked changes in the germinating condition of such seeds. On the other hand, seeds of burs buried beneath the soil have a very different environment. Their moisture content may be high and the compact soil about the burs in which the seeds are still encased, together with that imposed by the seed coat, may materially interfere with the gaseous ex-



change of the seeds. Under such conditions, seeds that failed for any reason to germinate in the spring, would not be likely to do so later in the season, unless by some disturbance of the soil, they should be brought under more favorable conditions for germination. It is well known that of the two seeds in the burs of *Xanthium* that have been covered with soil, frequently the so-called lower seed germinates the first spring after maturity while the upper seed remains until the following spring or even later before germination takes place. This behavior has been accounted for on the basis of the difference in the gaseous exchange between upper and lower seeds (1) and the surrounding medium due to a difference in permeability of seed coats and the ability of the lower seed to germinate with a slightly lower oxygen pressure than the upper seed (4).

By removing growing plants from the soil during the summer and fall and stalks of dead plants in the spring, burs were located, the lower seeds of which had given rise to plants, but which still contained the upper seeds ungerminated. The seed coats were removed from these seeds and the embryos along with the embryos of other seeds which had been stored in the laboratory were placed under germinating conditions. The results of the germination tests are given in table 2.

TABLE 2. *Germination of Embryos of Upper Seeds Which Were Taken from the Soil during the Summer and Fall after the Lower Seeds Had Given Rise to Plants*

No. of Experiment	No. Seeds Used	Time Collected	Germination—Days														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	10	July 1	1	1	0	3	1	3	0	0	0	0	1				
2	10	" 9	1	3	0	2	1	2	0	0	0	1					
3	18	" 25	1	8	2	5	1	0	0	0	0	0	1				
4	15	" 25	8	6	1												
5	20	Control	17	3													
6	20	"	20														
7	15	Sept. 7	0	1	0	0	0	1	3	2	4	1	1	1	0	0	1
8	10	March 5	9	1													
9	20	Control	20														

In experiments 1, 2, 3, and 4 the seeds were taken from a roadside where the soil was rather compact and dry at the time the seeds were obtained. In experiment 3 the seeds were kept intact in the germinator 48 hours, after which the seed coats were removed and the embryos were returned to the germinator. In experiment 4 the intact seeds were kept upon moist cotton in the refrigerator for a period of 30 days, when the seed coats were removed and the embryos were placed under the same germinating conditions as the others. In experiments 7 and 8 the seeds were obtained from a locality in which the soil was quite moist during the greater part of the year and at times was completely saturated.

In experiment 8 only a few seeds were procured from a large number of

seed stalks removed from the soil in March, due no doubt to a lack of development of a second seed in burs or to their destruction during the fall and winter by small rodents or insects. However, the rapidity or the vigor with which the naked embryos of these seeds that had passed through the winter beneath the soil germinated as compared with others under the same conditions, but collected in the fall, are doubtless characteristic of seeds that have overwintered in the soil.



TEXT FIG. 3. Embryos of cocklebur after 24 hours in germinator. A, after-ripened seeds; B, dry-stored seeds.

This shows clearly the tendency of non-germinating embryos within the seed coats and burs in the soil to go into a dormant condition during the warm weather of summer and to after-ripen, or go out of dormancy, during cool weather of winter. This development of embryo dormancy during the summer and after-ripening during the winter is probably a rather general phenomenon with non-germinating embryos of seeds of wild plants in the soil of the temperate zone. At least this rhythm has been shown in the preceding paper for seeds of *Ambrosia trifida* which have dormant embryos at maturity and in this paper for *Xanthium* seeds which have non-dormant embryos at maturity.

#### REMOVAL OF DORMANCY IN TREATED SEEDS

A condition of dormancy may not only be induced in the embryos of seeds of the cocklebur, but the dormant condition likewise may be removed. When the dormant seeds were placed upon moist cotton in petri dishes and kept at low temperature, preferably about 5° C., for several weeks, the

embryos gradually recovered from the dormant condition and germinated as readily or even more readily than embryos of dry stored seeds.

Text figure 3 *A* shows upper seeds of burs which were kept at high temperature in clay for four months when they were transferred to a temperature of 5° C. at which they were allowed to remain for three months. They were then taken from the cold, the seed coats removed, and the embryos placed under germinating conditions. The embryos were photographed 24 hours later. That the embryos had suffered no injury during the prolonged period at high temperature followed by an almost equal period in the cold is evident when the readiness and the energy with which they germinated are compared with conditions in embryos of dry stored seeds after a similar period in the germinator, as in text figure 3 *B*.

#### CATALASE ACTIVITY OF DORMANT AND AFTER-RIPENED SEEDS

The catalase activity of seeds of *Xanthium* both during the development of dormancy at high temperatures and the removal of dormancy at low temperatures corresponds to the results obtained in a study of the after-ripening and development of secondary dormancy in seeds of *Ambrosia trifida* (2).

When the seeds of *Xanthium* were prevented from germinating at temperatures at which they ordinarily germinate, through a restriction of the oxygen supply by means of clay or agar, the catalase activity decreased. Shull and Davis (5), working with *Xanthium* seeds with seed coats intact at temperatures below the minimum for germination of intact seeds, reported that at first there was a rise in the respiratory rate accompanied by a similar rise in the catalase activity, but after a period in the germinator there was a fall in each until the catalase activity was no greater than in air dry seeds.

In table 3 it will be observed that at temperatures considerably above the minimum for germination of intact seeds, but with the oxygen supply sufficiently restricted by means of either clay or agar to prevent germination, the catalase activity of seeds after from 50 to 60 days was even less than that of seeds in dry storage. The reduction of catalase of seeds with only the anterior portion embedded in clay as compared with that of the whole seed embedded in a coating of agar, is evidently due to the greater restriction of the oxygen supply to the more active portion of the embryo involving the hypocotyl and plumule. It also indicates that there is no very wide diffusion of oxygen through the embryo from the regions where it enters the seed. The respiration of intact dormant seeds was also less than that of untreated seeds. The catalase no doubt varies within certain limits with the oxygen supply to the embryo and there is a more or less close relation between the catalase activity and the restricted respiration of seeds at high temperatures as was formerly shown by Shull and Davis (5) for non-dormant seeds of *Xanthium* at temperatures below the minimum for germination.

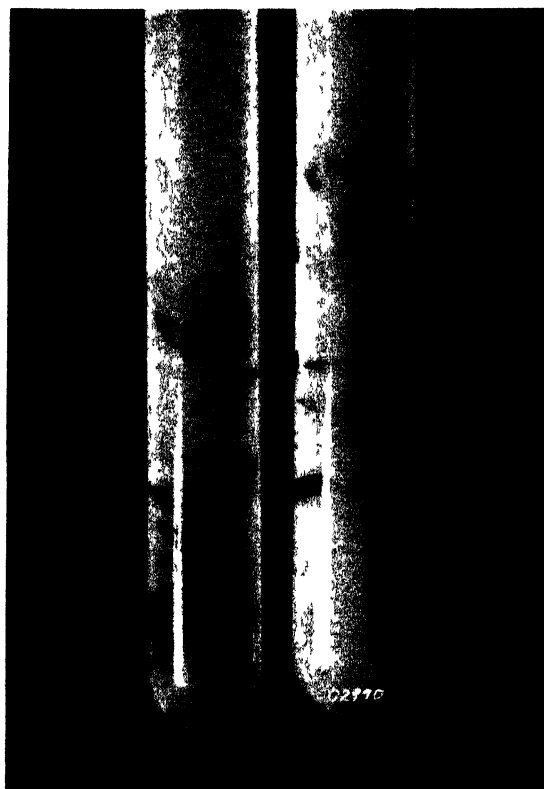
TABLE 3. *The Catalase Activity of Xanthium Seeds under Various Conditions*

No. of Seeds	Kind of Seeds	Treatment of Seeds	Wt. grams Soaked Seed	Cc. of O <sub>2</sub> Released after 10 Minutes	Av. cc. of O <sub>2</sub> Released per Seed
2	Upper	Seeds soaked overnight.		17.2	
2	"			17.0	
2	"		.40	16.5	8.45
2	"	Embedded in agar 60 days at temperatures 27°-29° C.		14.0	
2	"			13.7	
2	"		.41	14.5	7.00
2	"	Hypocotyl ends of seeds embedded in clay 60 days at temp. 27° to 29° C.		13.5	
2	"			10.1	
2	"		.42	11.5	5.8
2	"	Embedded in agar at high temperature 80 days, then removed from agar and placed in icebox 50 days.		23.5	
2	"			20.0	
2	"		.41	20.5	10.6
2	Lower	Seeds soaked overnight.		21.5	
2	"			23.7	
2	"		.52	23.5	11.4
2	"	Embedded in agar 80 days at 27°-29° C., then in icebox 40 days.		43.5	
2	"			47.6	
2	"		.56	43.0	22.3

## RELATION OF OXYGEN PRESSURE TO DORMANCY

The lower soaked seeds of burs were embedded near the bottom of large tubes of agar. The tubes were then sealed as shown in text figure 4. At this depth in the agar the oxygen supply to the embryos of the seeds must have been greatly reduced. Similar seeds in petri dishes were covered by a thin layer of agar. After 57 days at 26° to 30° C. both sets of seeds were taken from the agar, the seed coats were removed, and the embryos were placed under germinating conditions. They were photographed four days later. The results are shown in text figure 5 *A*, indicating the embryos of seeds taken from tubes of agar, and *B*, those taken from seeds in petri dishes (text fig. 1 *B*). The slight dormancy of the embryos taken from the tubes and the very pronounced dormancy in those taken from petri dishes can be accounted for only by the difference in the gaseous exchange of the seeds, resulting from the different depths at which they were embedded in the agar.

Seeds sealed in agar in tubes were later kept 100 days at a temperature of 30° C. without any apparent sign of dormancy in the embryos. Seeds embedded in agar at the bottom of tubes always decayed if the agar withdrew from the sides of the tubes so as to admit air.



TEXT FIG 4. Method of embedding seeds deeply in agar

It is probable that seeds of *Xanthium* can be kept indefinitely in a medium to which little air is admitted, provided the medium does not keep them in a so fully saturated condition as does agar, in which extreme care is necessary to prevent spoiling.

The above experiments seem to indicate that the development of dormancy, or at least the rate of development, is closely associated with the rate of oxygen supply to the embryo. Below a certain rate of oxygen supply the changes that take place during the development of dormancy either do not take place or are greatly slowed down. The same characteristic was found true of seeds of *Ambrosia* (2), but seeds of *Ambrosia*

will not stand the prolonged high temperatures to which seeds of *Xanthium* may be subjected. It was suggested in the case of *Ambrosia* that possibly a rate of oxygen supply just below that which at a given temperature is



TEXT FIG. 5. Germination of embryos after four days when taken from seeds made partly dormant by covering with agar. A, a thin layer of agar in a petri dish; B, buried deeply in agar in a test tube.

necessary to produce germination, is near the optimum for the development of dormancy.

#### SUMMARY

1. The naked embryos of seeds of *Xanthium* at maturity show no dormant tendencies, but dormancy may be induced in the embryos of intact seeds at temperatures at which germination ordinarily takes place, provided the restriction of the gaseous exchange by the seed coats is supplemented by means of clay or agar to a point where germination may not take place.

2. The time required for the development of dormancy in the embryos of intact seeds varies from two to several months and no doubt depends upon the temperature and on the magnitude of the restriction of the gaseous exchange.

3. During the period in which dormancy is induced there is a perceptible drop in both the catalase activity and the respiratory rate of the seed.

4. The dormant embryos of seeds of *Xanthium* after the removal of seed coats usually exhibit under germinating conditions a marked variation in the dormancy of the various embryos themselves. This is very likely due to differences in the permeability of the seed coats to gases during the period in which dormancy was induced.

5. When seeds, the embryos of which have been rendered dormant were kept moist and at low temperatures, preferably about 5° C., the dormancy after a time disappeared from the embryos as was indicated by the rapidity or vigor of the germination of the naked embryos at suitable temperatures.

6. During the period of after-ripening or the removal of dormancy at low temperature, there was a rise in the catalase activity and also in the respiratory intensity of the seed.

7. Seeds of burs buried in the soil where they have a high moisture content apparently become more or less dormant during the summer and in turn lose this dormancy during the low temperatures of the succeeding winter and spring.

8. An oxygen supply to the seed just below that necessary to cause germination at a rather high temperature appears best for the development of dormancy.

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## HASTENING THE GERMINATION OF SOME CONIFEROUS SEEDS

LELA V. BARTON

Germination experiments on southern pine seeds (*Pinus taeda*, *Pinus echinata*, *Pinus caribaea*, and *Pinus palustris*) conducted in this laboratory in 1928 (Barton, 1) have been extended to include seeds of a number of different species of pine together with several other conifers. It seemed desirable to ascertain the response of these different forms to low temperature stratification which proved effective in hastening the germination of the southern pines.

The present paper reports results of these tests on both 1927 and 1928 crops of seed. Of the 1927 crop the seeds tested were: *Pinus austriaca*, *Pinus Banksiana*, *Pinus Cembra*, *Pinus densiflora*, *Pinus excelsa*, *Pinus flexilis*, *Pinus insignis*, *Pinus Laricio*, *Pinus Lambertiana*, *Pinus monticola*, *Pinus contorta Murrayana*, *Pinus ponderosa* I, *Pinus ponderosa* II, *Pinus resinosa*, *Pinus Strobus*, and *Pinus Thunbergii*. All of these seeds except those of *Pinus flexilis*, were obtained from Thomas J. Lane, seedsman, and were received in this laboratory in July 1928, when germination tests were started. They were collected in the fall of 1927 and presumably were kept in dry storage at room temperature up to the time of shipment.

The 1928 crop of seeds included *Abies arizonica*, *Cupressus macrocarpa*, *Libocedrus decurrens*, *Picea canadensis*, *Picea excelsa*, *Picea Omorika*, *Picea pungens*, *Picea sitchensis*, *Pinus austriaca*, *Pinus Banksiana*, *Pinus Cembra*, *Pinus contorta*, *Pinus contorta Murrayana* I, *Pinus contorta Murrayana* II, *Pinus contorta Murrayana* III, *Pinus Coulteri*, *Pinus densiflora*, *Pinus excelsa*, *Pinus flexilis*, *Pinus insignis*, *Pinus koraiensis* I, *Pinus koraiensis* II, *Pinus Lambertiana* I, *Pinus Lambertiana* II, *Pinus monticola*, *Pinus ponderosa*, *Pinus resinosa*, *Pinus rigida*, *Pinus Strobus*, *Pinus Thunbergii*, *Sciadopitys verticillata*, *Sequoia sempervirens*, *Taxodium distichum*, *Thuya gigantea*, *Thuya occidentalis*, and *Thuya orientalis*. *Pinus contorta Murrayana* I and *Pinus flexilis* (1927 and 1928 crops) were furnished through the courtesy of the U. S. Dept. of Agriculture Forest Service, Rocky Mt. Experiment Station, Colorado Springs, Colo. All of the other 1928 seeds were obtained from Thomas J. Lane, and most of them were received in this laboratory in November 1928.

### METHOD

The method was essentially the same as that described in a previous paper (Barton, 1). Preliminary to germination experiments an examination

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of embryos was made by cutting the seeds. This process is usually called a cutting test of the embryos. These tests served as a basis for calculating "real" percentages of germination (Jacobs, 2), but they should not be considered as absolute indices of the germination capacity of the seed lot because of the limited number of seeds available for cutting. In the fresh seeds, however, there seemed to be, for the most part, a close correlation between the embryo tests and the seedling production in the soil.

The seeds were mixed with moist acid peat and placed in ovens at 0°, 5°, and 10° C. for different periods of time. They were aerated and moistened at intervals of 6 to 10 days throughout the test. In special instances other temperatures than 0°, 5°, and 10° C. were used. Some of the seeds which were very slow in germinating were given additional treatment of 15° C. as well as weekly alternating temperatures of - 5° to 5° C. and 5° to 10° C. A number of the slow germinating seeds (1928 crop) were also planted in flats and put outside in cold frames in open, mulched, and board-covered soil in December, 1928.

The number of seeds stratified depended upon the quantity available but where possible at least 600 seeds were used. Samples (100 seeds each if possible) were taken from these seeds at intervals of one, two, three, and in a few cases four or more months, and planted at a depth of 1/8 to 1/4 inches in a greenhouse in flats containing equal amounts of sand, peat, and wood soil. Samples of seeds which had been kept dry at room temperature were planted at the same time for controls.

The seedlings were counted as soon as they appeared above ground, and as a general rule they were discarded after counting. The word "germination" as it is used throughout this paper in referring to greenhouse plantings means the appearance of seedlings above ground. In describing cultures in the separate ovens the word refers to the appearance of the primary root.

Apparent germination percentages are those calculated on the basis of the number of seeds planted. "Real" germination percentages are those calculated on the basis of the number of sound embryos in the seeds used. Apparent germination percentages are used in the results and discussion except in special instances.

## RESULTS AND DISCUSSION

### **Pinus**

#### *Pinus austriaca*

From table 1 it will be seen that in the 1927 crop the cutting tests revealed 98 percent good embryos. A considerable number of these "good" embryos appeared yellow and rancid and this may account for the small germination percentages obtained. This same statement may be taken to apply in a greater or less degree to all of the 1927 seeds. This is not surprising in view of the fact that all of these seeds were practically a year

old before they were received and the vitality of most pine seeds decreases rather rapidly with ordinary storage.

TABLE I. *Results of Embryo Tests*

Species	Crop	Number of Seeds Examined	Percent Good Embryos
<i>Abies arizonica</i> .....	1928	100	69
<i>Cupressus macrocarpa</i> .....	1928	100	59
<i>Libocedrus decurrens</i> .....	1928	50	40
<i>Picea canadensis</i> .....	1928	100	74
<i>Picea excelsa</i> .....	1928	100	96
<i>Picea Omorika</i> .....	1928	100	81
<i>Picea pungens</i> .....	1928	100	93
<i>Picea sitchensis</i> .....	1928	100	79
<i>Pinus austriaca</i> .....	1927	100	98
<i>Pinus austriaca</i> .....	1928	100	95
<i>Pinus Banksiana</i> .....	1927	100	97
<i>Pinus Banksiana</i> .....	1928	100	90
<i>Pinus Cembra</i> .....	1927	100	77
<i>Pinus Cembra</i> .....	1928	50	92
<i>Pinus contorta</i> .....	1928	100	99
<i>Pinus contorta Murrayana</i> .....	1927	100	98
<i>Pinus contorta Murrayana</i> , I.....	1928	100	98
<i>Pinus contorta Murrayana</i> , II.....	1928	100	99
<i>Pinus contorta Murrayana</i> , III.....	1928	100	98
<i>Pinus Coulteri</i> .....	1928	25	96
<i>Pinus densiflora</i> .....	1927	100	96
<i>Pinus densiflora</i> .....	1928	100	94
<i>Pinus excelsa</i> .....	1927	100	80
<i>Pinus excelsa</i> .....	1928	100	70
<i>Pinus flexilis</i> .....	1927	100	83
<i>Pinus flexilis</i> .....	1928	50	54
<i>Pinus insignis</i> .....	1927	100	97
<i>Pinus insignis</i> .....	1928	100	93
<i>Pinus koraiensis</i> , I *.....	1928	—	—
<i>Pinus koraiensis</i> , II.....	1928	25	100
<i>Pinus Lambertiana</i> .....	1927	22	100
<i>Pinus Lambertiana</i> , I.....	1928	25	88
<i>Pinus Lambertiana</i> , II.....	1928	50	78
<i>Pinus Laricio</i> .....	1927	100	88
<i>Pinus Laricio</i> .....	1928	100	100
<i>Pinus monticola</i> .....	1927	100	79
<i>Pinus monticola</i> .....	1928	100	95
<i>Pinus ponderosa</i> , I.....	1927	100	87
<i>Pinus ponderosa</i> , II.....	1927	100	94
<i>Pinus ponderosa</i> .....	1928	100	85
<i>Pinus resinosa</i> .....	1927	100	97
<i>Pinus resinosa</i> .....	1928	100	95
<i>Pinus rigida</i> .....	1928	100	91
<i>Pinus Strobus</i> .....	1927	100	96
<i>Pinus Strobus</i> .....	1928	100	98
<i>Pinus Thunbergii</i> .....	1927	100	85
<i>Pinus Thunbergii</i> .....	1928	100	79
<i>Sciadopitys verticillata</i> .....	1928	100	90
<i>Sequoia sempervirens</i> .....	1928	300	11.3
<i>Taxodium distichum</i> .....	1928	200	76
<i>Thuja gigantea</i> .....	1928	100	80
<i>Thuja occidentalis</i> .....	1928	100	55
<i>Thuja orientalis</i> .....	1928	100	74

\* Not enough seeds to make cutting test.

The highest germination from treated seeds of *Pinus austriaca* 1927 was 16 percent which was obtained in 14 days after planting seeds which had been stratified for two months at 5° C. The average germination of the untreated seeds was 20 percent in 60 days.

Of the 1928 lot of seeds, 95 percent had good embryos and much higher germination percentages were obtained. Here the controls averaged 47 percent in 24 days. Approximately the same results were obtained from one, two, or three months' stratification at either 0° or 5° C. Seeds from these conditions gave about 50 percent germination in 12 to 18 days after planting in the greenhouse. Hence it might be said that stratification is effective in producing prompt germination of *Pinus austriaca* seeds but there is no appreciable increase in the number of seedlings.

#### *Pinus Banksiana*

Tozawa (7) reports 94 to 100 "real" percent germination for this species after the seeds had been subjected to "exposed burying storage" for a period of about four months.

Experiments in this laboratory did not give such high germination percentages (tables 2 and 3). Results indicated that the beneficial effects of stratification in this case were to be found only in the hastening of germination since the average final germination percentage of the controls was practically as high as that of the treated seeds. It would seem advisable, however, to stratify these seeds at 0° or 5° C. for a period of two months prior to planting since it makes a difference of 10 to 20 days in appearance of seedlings.

The 1928 crop of seeds showed a slight increase in germination over the 1927 crop but this difference was not so marked as in the case of *Pinus austriaca*.

#### *Pinus Cembra*

Although germination tests were made on both 1927 and 1928 lots of these seeds, no seedlings were obtained. The cutting tests revealed many embryos which apparently were sound but which had a wrinkled and rather dried appearance. It is possible that none of these seeds were viable, but it is very likely that favorable after-ripening conditions have not yet been found.

Kienitz (3) found that Cembra pine under the most favorable conditions in a seed bed rarely germinates the first year but there is an abundant germination the second year. Zederbauer (9) also reports that *Pinus Cembra* and *Pinus koraiensis* proved especially hard to germinate. It is certain that the germination of *Pinus Cembra* seeds will require a great deal more study.

#### *Pinus contorta*

Of this species Toumey and Stevens (6) say: "The Pacific coast form of this species and the Rocky Mountain form known as lodgepole pine show,

in the nine tests from seeds collected in different regions and at different times, a remarkably low germination within the period of the test. In one sample but 14 percent germinated within the period of 50 days, yet cutting tests at the time of termination of the experiment showed that 80 percent of the ungerminated seeds were sound. The average germination in 50 days was only 11.2 percent and the highest 24 percent."

All the seeds available for this study were of the 1928 crop. Ninety-nine percent of the seeds were apparently good. The response to stratification was definite and favorable. The optimum temperature for stratification seemed to be 5° C. and the time two months. Seeds planted after this treatment gave 86 percent germination in 18 days whereas the average for the checks gave 51 percent in 28 days.

#### *Pinus contorta Murrayana*

Although 98 percent of the 1927 crop of the seeds of this species contained embryos, the results of the germination tests indicated very low vitality, especially so when compared with the results of three lots of the same kind of seed from the 1928 crop. The highest germination percentage (6 percent) was obtained from seeds which had been stratified for two months at 5° C. The check lot of seeds averaged four percent germination so it cannot be said that stratification had any appreciable effect.

There was, however, a decidedly favorable response to stratification in the 1928 seeds of which there were three different lots, numbers I, II, and III. Ninety percent of the seeds of number I germinated in 14 days after two months' stratification at 5° C. while the average control for these same seeds gave 63 percent germination in 24 days. Similar results are to be found in the cases of numbers II and III.

For *Pinus contorta Murrayana*, then, stratification of the seeds at either 0° or 5° C. for one, two, or three months is decidedly beneficial in the production of prompt and complete stands of seedlings.

#### *Pinus Coulteri*

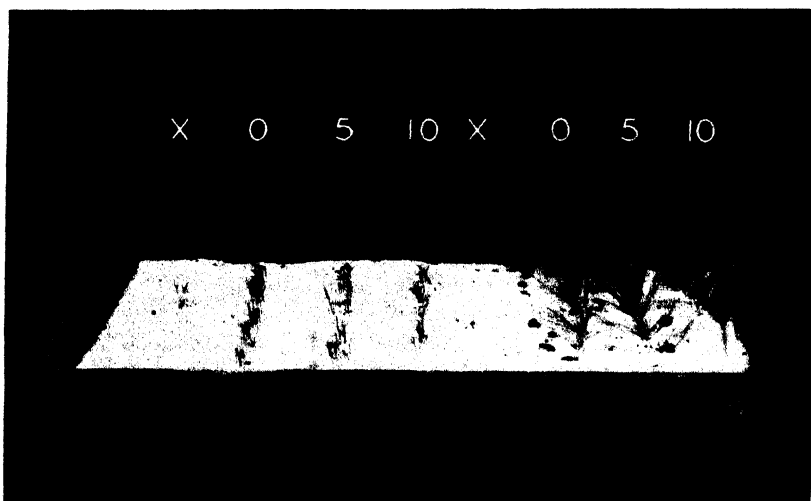
Only a limited number of the 1928 crop of these seeds was available and yet they furnished one of the most striking examples of low temperature stratification effects.

The sample plantings were of ten seeds each. Seeds which had been stratified at 0°, 5°, or 10° C. for one, two, or three months germinated to the extent of 80 to 100 percent in 19 to 25 days after planting in a flat in the greenhouse (text fig. 1, right). None of the untreated seeds had germinated at the end of 50 days.

#### *Pinus densiflora*

In this instance there was very little difference in the results of the germination tests on 1927 and 1928 seeds. The cutting tests also showed that the two lots were of practically equal vitality.

Stratification for two months at  $0^{\circ}$  or  $5^{\circ}$  C. proved effective in hastening germination. The seeds left at these temperatures longer than three months began to germinate. This was especially true of the 1928 seeds. A small percentage germinated at  $10^{\circ}$  C. before they had been in this condition one month. However, a sample planting of 1928 seeds after stratification for one month at  $10^{\circ}$  C. resulted in the best germination percentage (82) obtained in these tests. Hence it would seem that there would be very little if any loss due to seedling production in the oven at  $10^{\circ}$  C. and at the same time good results could be obtained from greenhouse plantings after one month at this temperature. It would be impractical to stratify at  $10^{\circ}$  C. for a longer period.



TEXT FIG. 1. Seeds were stratified for one month at  $0^{\circ}$ ,  $5^{\circ}$ , and  $10^{\circ}$  C. Picture taken 25 days after planting. Left to right: *Thuya gigantea*; control,  $0^{\circ}$ ,  $5^{\circ}$ , and  $10^{\circ}$  C., *Pinus Coulteri*; control,  $0^{\circ}$ ,  $5^{\circ}$ , and  $10^{\circ}$  C.

Tozawa (7) reports 98 to 100 percent "real" germination of *Pinus densiflora* after four or five months' "exposed burying storage" or "indoor burying storage." He also obtained 99 to 100 percent "real" germination from one month (March) "exposed burying storage" and from dry storage. However, he found that the seeds with four or five months' treatment germinated more readily.

#### *Pinus excelsa*

This species of pine proved one of the most difficult to germinate. The best germination was eight percent in 25 days. This resulted from a planting of 1928 seeds which had been stratified at  $10^{\circ}$  C. for three months. In addition to the usual tests after one, two, and three months at  $0^{\circ}$ ,  $5^{\circ}$ ,

and 10° C., plantings of the 1927 seeds were made after four and eight months at 5° C. There was no germination from the former and only one percent in 15 days from the latter. Seeds of the 1928 crop were tested after four months at 0°, 5°, and 10° C. Those from 0° C. germinated to the extent of two percent 25 days after planting in the greenhouse.

Additional seeds of the 1928 crop were stratified at an alternating temperature of - 5° to 5° C. and at a constant temperature of 15° C. for periods of one, two, three, and four months. Sample plantings were made from each temperature at the end of each of these periods but no seedlings were produced.

Since the cutting tests revealed 80 and 70 percent good embryos in the 1927 and 1928 lots, respectively, it is evident that the methods here reported are unsatisfactory for the treatment of seeds of *Pinus excelsa*.

#### *Pinus flexilis*

A reference to table 1 will show that in this case the 1927 seeds were better than the 1928. In either case the seeds may be left at 0°, 5°, or 10° C. for one, two, or three months. The response of the 1927 seeds to any of these conditions was very marked. For instance, after two months at 5° C., a sample planting gave 100 percent germination 18 days after planting, while the control produced 50 percent germination in 40 days.

The stratification effects were not so striking for the 1928 seeds (tables 2 and 3). Here the principal result was the shorter germination period of treated seeds, as the final germination percentages from untreated seeds approximated the number produced from treated seeds.

#### *Pinus insignis*

As would be expected from the results of the cutting tests (table 1) 1927 and 1928 crops of these seeds behaved in a similar manner. For both of them 5° C. for two or three months was favorable for after-ripening. Seeds thus treated produced a rather complete stand of seedlings (75 to 84 percent) in 14 to 18 days while the untreated seeds required 50 to 70 days for a smaller percentage germination (69 percent).

#### *Pinus koraiensis*

No 1927 seeds of this pine were available but experiments were performed with two different lots of 1928 seeds. From table 3 it will be seen that the first lot (*Pinus koraiensis* I 1928) gave 20 percent germination within 25 days after planting when the seeds had been stratified for three months at 5° C. Seeds of *Pinus koraiensis* II 1928, however, did not germinate at all when planted after stratification at 0° or 5° C. for one, two, or three months, but produced seedlings to the extent of ten percent after stratification for one or two months at 10° C. When these seeds were planted after stratification for five months at 0° or 5° C., 33 percent of

them germinated in 43 days. This seems to point to the need of a long stratification period.

Seeds of *Pinus koraiensis* were also stratified for one, two, and three months at a constant temperature of 15° C. and at a weekly alternating temperature of - 5° to 5° C. Results of sample plantings showed these temperatures to be of little value for stratification.

In all sample plantings of stratified seeds of this species only ten seeds were used in each lot.

Samples of one hundred seeds each of *Pinus koraiensis* I 1928 were planted in flats and put in cold frames in mulched, board-covered, and open soil on December 10, 1928. On July 2, 1929, seeds held under the three conditions had germinated to the extent of 12, 21, and 6 percent, respectively.

Further experiments on *Pinus koraiensis* are now being planned.

### *Pinus Lambertiana*

In spite of the fact that embryo tests of *Pinus Lambertiana* II 1928 showed that the seeds of this lot were inferior to those of *Pinus Lambertiana* I 1928 or of *Pinus Lambertiana* 1927 (table 1), the highest germination percentage (92) was obtained with these seeds after they had been stratified for three months at 10° C. Each of the three lots, however, showed the marked effect of low temperature stratification as an effective agent for hastening germination. This fact is clearly shown in tables 2 and 3 and is significant in view of the difficulties in germination reported by Toumey and Stevens (6). They say that the earliest germination attained in any of the tests was 20 days. In some of their samples there was no germination in 50 days. The germination in 50 days averaged but eight percent.

Jacobs (2) reports the normal germination of sugar pine based on 12 tests for 120 days as varying from 18 to 53 percent. Soaking for four days and exposure to freezing for 48 hours he found most favorable to induce the early and complete sprouting of sugar pine seeds. The beneficial action of soaking, Jacobs considered due to the action of bacteria in tap water exposed to air. This is recommended as a possible means for obtaining a high percentage of vigorous seedlings in nursery beds. He found soaking for four days most beneficial of all pretreatments tried and in his lots 1 and 4 the germination reached 80 and 73 percent ("real" germination percent), respectively, in a period of 20 days. He used fresh seeds, that is, extracted seeds which had been dried for one week at an average room temperature of 22° C. before being used for germination tests.

Stratification effects on *Pinus Lambertiana* II 1928 here reported compare favorably with the germination results of Jacobs (2). After three months at 0°, 5°, and 10° C. these seeds produced seedlings to the extent of 92, 85, and 118 "real" percent, respectively (table 2). A "real" percentage of over 100 is due either to the planting of seeds which have more than the

average number of good embryos or to the selection of poor seeds for the cutting tests.

*Pinus Laricio*

For the 1927 seeds one or two months at either 0° or 5° C. puts the seed in condition for sprouting. Here the beneficial effect of low temperature treatment is to be found only in the earlier stands of seedlings since the untreated seeds germinated equally well but in 60 days as compared to 14 to 18 days for the treated seeds.

The 1928 seeds were not received until May 1929, and seem to have poor germination quality (tables 2 and 3). However, the stratification effects are noticeable (table 3).

*Pinus monticola*

Of the seeds of this species Toumey and Stevens (6) say: "The earliest germination was in 25 days. The highest number of sound ungerminated seeds after 50 days was 48 percent. In this sample no seeds germinated within the 50-day period. Taking these samples as a whole they show a remarkably low germination capacity. Furthermore, the germination in 50 days averaged but 2.7 percent."

Wahlenberg (8) has worked with these seeds and reports that fall sowing of western white pine results in prompt and complete germination the following spring. Larsen (4) reports the same beneficial effect of fall sowing.

The 1927 and 1928 lots of seeds used in the present study had 79 percent and 95 percent good embryos. Consequently there should have been a better yield of seedlings from untreated seeds than that reported by Toumey (6). We have obtained similar results. The average percentage obtained after 50 days in the 1927 lot was 11 and that obtained from the 1928 seeds was ten in the same period.

In the 1927 seeds, the best germination obtained was 48 percent. This resulted 70 days after planting seeds which had been stratified for three months at 5° C. However, the major portion of these seedlings had been produced at the end of 37 days.

The best germination obtained in the 1928 seeds (30 percent 23 days after planting) was the result of three months' stratification at 0° C.

Four and five months' stratification at 0°, 5°, and 10° C. as well as one, two, three, and four months at a weekly alternating temperature of 5° to 10° C. were also tried with the 1928 seeds. None of these results were superior to those already given.

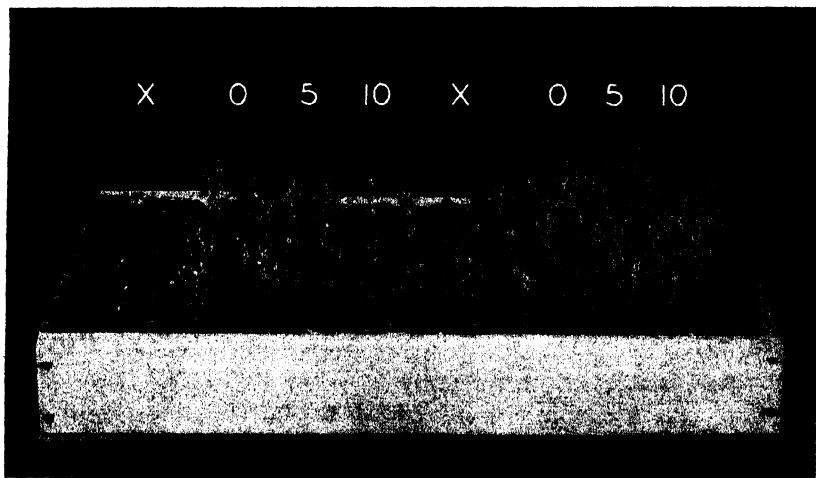
Three flats of 1000 seeds each were planted and put in cold frames in mulched, board-covered, and open soil December 10, 1928. On July 2, 1929, these flats showed germinations of six, eight, and seven percent, respectively. The number of seedlings obtained in this experiment was unduly low because part of the seeds were eaten by mice.



*Pinus ponderosa*

Toumey and Stevens (6) report an average germination of 43 percent in 50 days with the earliest germination within ten days after starting the tests. They found that some samples attained a germination as high as 22 percent in ten days and that the germination energy period was usually within 25 days and sometimes within 15 days after seeding (text fig. 2, right).

Both lots of 1927 seeds used in the present experiments yielded better productions of seedlings than the 1928 seeds. In general *Pinus ponderosa*



TEXT FIG. 2. Seeds were stratified for two months at 0°, 5°, and 10° C. Picture taken 19 days after planting. Left to right: *Pinus resinosa* 1928; control, 0°, 5°, and 10° C. *Pinus ponderosa* 1928; control, 0°, 5°, and 10° C.

seeds germinate readily without treatment of any kind. In every case the untreated seeds had begun to germinate in ten days and in one case (*Pinus ponderosa* II 1927) the germination within this period amounted to 36 percent. This is in agreement with Toumey's report as is the fact that the germination is practically complete within 20 to 30 days. However, a higher final germination percentage (50 to 79 percent) was obtained in the present experiments.

Stratification for one or two months at either 0° or 5° C. has the advantage that a higher germination percentage (74 to 97 percent) is promptly obtained (tables 2 and 3).

*Pinus resinosa*

Both the 1927 and 1928 seeds responded favorably to stratification for one, two, or three months at 0°, 5°, or 10° C. Very good germination (from 73 to 95 percent within a period of 14 to 25 days) was obtained in

all cases (text fig. 2, left). However, the control lots of seed also produced good stands of seedlings (64 to 67 percent in 20 to 24 days).

In tests on *Pinus resinosa*, Toumey and Stevens (6) obtained an average germination of 33.6 percent in 50 days. They also report that taking the samples of this species as a whole, the peak of germination was attained in 25 days or less.

#### *Pinus rigida*

Only 1928 seeds of this species were tested. Table 3 reveals very clearly the beneficial effect of stratification. Here it is seen that one month at 5° C. gave as good results as two or three months. Seeds planted after one month at 5° C. gave 87 percent germination after 12 days and 95 percent after 18 days and if the experiment was extended to 40 days the percentage germination reached 99. This is in sharp contrast to the untreated seeds of which only three percent had germinated after 12 days and which reaches 33 percent after 50 days (text fig. 3).

Very good germination (79 to 96 percent) was also obtained after one, two, or three months at 0° or 10° C. (table 2).

#### *Pinus Strobus*

The seeds of this species proved rather difficult to germinate. In spite of this fact, however, the low-temperature effects were quite marked and especially so in the 1927 seeds. For instance after two months at 5° C., 69 percent of the seeds had germinated 24 days after planting while the corresponding control showed three percent germination 60 days after planting (see table 3).

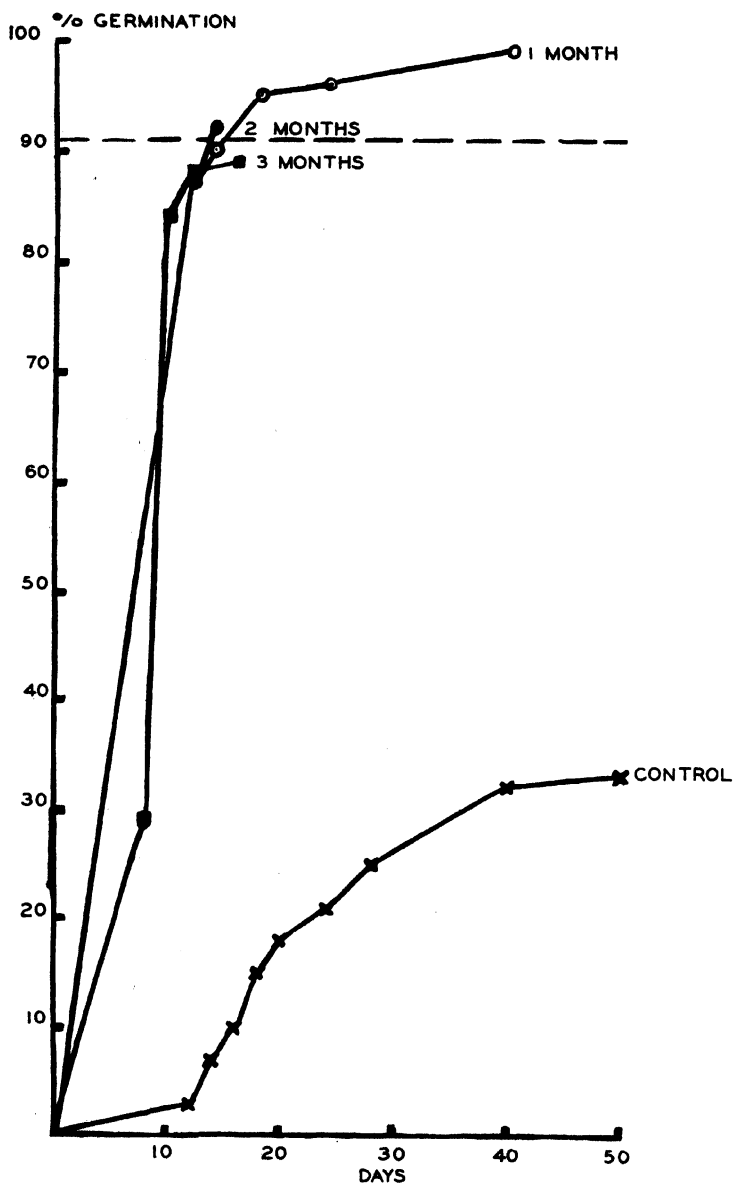
After the same stratification period the 1928 seeds attained 26 percent germination in 24 days, while the corresponding control yielded four percent 28 days after planting.

Seeds were also stratified at a weekly alternating temperature of - 5° to 5° C. as well as a constant temperature of 15° C. From these stratifications sample plantings were made after one, two, three, and four months. The results showed these temperatures inferior to those of 0°, 5°, and 10° C. or after-ripening.

*Pinus Strobus* seeds have been reported as being very irregular in germination and as being greatly benefited by fall sowing (Toumey, 6), which practice is, of course, essentially the same as low-temperature stratification.

According to Schmidt (5), *Pinus Strobus* stored dry will not germinate without soaking in water. He advises storage in a cool, moist room for 30 days after a swelling period of 16 hours. As a result of treatment with a 16-hour water bath, he reports 78 percent germination. He also soaked the seeds in one percent hydrogen peroxid for 16 hours. From these seeds he obtained 81 percent germination in 60 days.

## PINUS RIGIDA



TEXT FIG. 3. The effect of stratification at 5° C. for one, two, and three months on germination of the seeds of *Pinus rigida*. Dotted line shows the percentage of good seeds as revealed by embryo tests.

*Pinus Thunbergii*

Seeds of both 1927 and 1928 crops proved difficult to germinate. The highest germination obtained was 20 percent. Five degrees C. proved as good as any temperature for after-ripening these seeds. When one compares the percentage and time of germination of the stratified and untreated seeds (table 3), the benefit of the low-temperature treatment is apparent. However, it leaves much to be desired.

One thousand seeds each were planted in three flats which were put in cold frames December 10, 1928, in mulched, board-covered, and open soil. On July 2, 1929, seedlings had been produced to the extent of two, four, and five percent, respectively.

**Other Coniferae***Abies arizonica*

Cutting tests showed that 69 percent of the seeds had good embryos. The best germination was 43 percent which was obtained 41 days after planting seeds which had been stratified for one month at 0° C. (table 2). However, seeds which had been stratified for two months at 5° C. produced seedlings to the extent of 36 percent within 14 days after planting. Since the control gave only two percent germination after 50 days, the beneficial effect of low-temperature treatment is evident.

*Cupressus macrocarpa*

Toumey and Stevens (6) found that the highest germination in 50 days was 17 percent and the average 9.7 percent. They report the highest germination capacity as 37 percent and the average only 16.2 percent.

In spite of the fact that cutting tests showed a germination capacity of 59 percent, the average germination of untreated seeds in the present tests was only six percent after 50 days (table 3). However, higher percentages were obtained after low temperature treatment. The best germination was 22 percent which resulted 23 days after planting seeds which had been stratified at 0° C. for two months. The best stratification temperature tried for these seeds was 0° C. (table 3).

*Libocedrus decurrens*

A small number of 1928 seeds of this species were received in May 1929. Cutting tests revealed only 40 percent good embryos.

The highest germination obtained was 23 percent (58 percent real germination) which resulted 24 days after planting seeds which had been stratified for one month at 0° C. These seeds also responded well to two months' stratification at either 0° or 5° C. (table 2). In any case low-temperature treatment had a marked effect since the control lot of seeds had germinated to the extent of only one percent in 50 days.

*Picea canadensis*

Stratification at 0° C. proved more favorable for after-ripening these seeds than at 5° C. while 10° C. could not be used at all because too many seeds (16.4 percent) germinate within a month at this temperature. From table 2 it will be seen that the highest germination percentage (96 percent in 25 days) was obtained after the seeds had been kept for two months at 0° C. The untreated seeds germinated to the extent of 48 percent in 26 days (table 3). Toumey and Stevens (6) report 37 percent as the highest germination percentage obtained in 50 days.

Only 1928 seeds of this species were available for the present tests.

*Picea excelsa*

The response of these seeds to low temperature stratification was essentially the same as that of *Picea canadensis* (tables 2 and 3).

*Picea Omorika*

In this species stratification for one or two months at 0°, 5°, or 10° C. appears to be equally good. The highest germination was 66 percent within 15 days after planting. In view of the fact that cutting tests revealed only 81 percent good embryos, the above percentage represents fairly complete germination. The average control gave ten percent germination in 16 days.

*Picea pungens*

In this case one month's treatment at 0°, 5°, or 10° C. is sufficient to give a stand of seedlings of from 74 to 80 percent in 16 days (table 2). The germination of the untreated seeds was only 47 percent in 50 days.

*Picea sitchensis*

This species proved more difficult to germinate than the other species of *Picea* included in this study. Of these seeds Toumey and Stevens (6) observed that germination seldom gets well under way for a period of 20 to 30 days and usually many sound seed remain ungerminated after a period of 50 days.

This same observation was made in the present study. However, since seeds which had been stratified for two months at 5° C. yielded 24 percent seedlings in 27 days and the control yielded only four percent in 40 days, again we can say that although we do not obtain a complete stand of seedlings the beneficial effect of stratification is evident. The germination tests were allowed to continue for 60 days.

*Sciadopitys verticillata*

*Sciadopitys verticillata* seeds were obtained from Conyers B. Fleu, seedsman, and were received in this laboratory January 2, 1929. The seeds were of the 1928 crop and were originally from Japan.

Germination tests of these seeds were made preliminary to storage tests which are now in progress. Oven tests of 200 seeds each were made in peat at constant temperatures of 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. as well as daily alternating temperatures of 10° to 30° C., 15° to 30° C., 20° to 30° C., and 20° to 35° C. The best germination temperature proved to be 20° C. where a percentage of 52.5 was obtained in 61 days with a final percentage of 60.5 in five months. The period of these tests was seven months.

Stratification of these seeds differed from that of any other seeds in this report in that three different media (peat, leached peat, and muck) were used. One thousand seeds each were placed in each medium at constant temperatures of 0°, 5°, 10°, and 15° C. as well as daily and weekly alternating temperature of 5° to 10° C. At the same time (January 1929) 1000 seeds each were planted in five flats, one of which was placed under each of the following conditions: 1, open cold frame; 2, mulched cold frame; 3, board-covered cold frame; 4, lowest temperature greenhouse available (40°-45° F.); and 5, highest temperature greenhouse available (65°-70° F.). The greenhouse temperatures increased, of course, as the season advanced.

Sample plantings from all the stratifications were made after two, three, four, five, and six months. The seedling productions from 0°, 5°, and 10° C. for one, two, and three months are shown in table 2. The best stratification conditions found were 10° C. for one or two months, or 0° or 5° C. for two months. No seedlings have as yet been obtained from the fourth, fifth, or sixth month's planting. However, since the seedlings do not begin to appear until 60 to 70 days after planting and since these last plantings were made in June, July, and August, 1929, it is possible that the seedling production will indicate some advantage in stratification.

In view of the results of the tests it would seem that stratification has no particular effect in hastening the germination of seeds of *Sciadopitys verticillata* since the untreated seeds grow just about as well as the treated ones (table 3).

Of the seeds which were planted in flats, those in the warm greenhouse germinated first. Here the germination reached 13.6 percent in three and one-half months and in four months the percentage was 27.3. After four months this flat was badly infected with damping-off which doubtless precluded additional germinations. Seedlings appeared in the low-temperature greenhouse after seven months (7.5 percent), while the seeds in the cold frames had produced no seedlings in this period.

#### *Sequoia sempervirens*

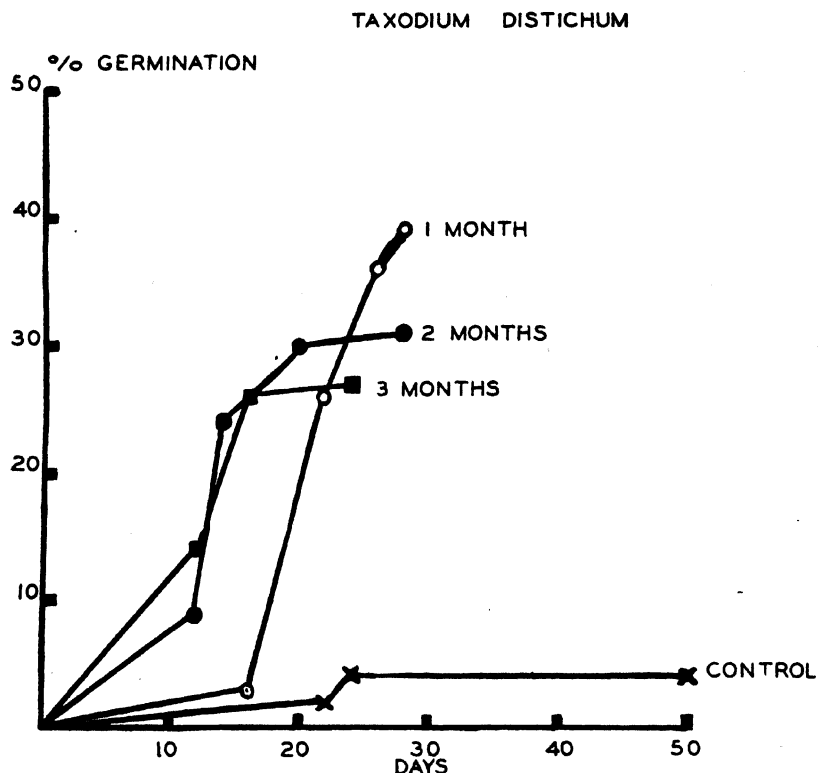
A lot of 1928 seeds of this tree were exceptionally poor in quality. Three hundred seeds were cut open and 88.7 percent of them were empty or molded, only 11.3 percent appearing good.

The usual stratification and sample plantings were made but no seedlings

were obtained. Toumey and Stevens (6) obtained as high as 21 percent germination in 50 days and an average germination of 8.8 percent.

*Taxodium distichum*

The germination capacity of these seeds as shown by the embryo tests was 76 percent (table 1). Stratification for one month at 5° C. seemed



TEXT FIG. 4. The effect of stratification at 5° C. for one, two, and three months on germination of the seeds of *Taxodium distichum*.

satisfactory for after-ripening the seeds. From seeds thus treated a seedling production of 39 percent was obtained in 28 days. The corresponding untreated seeds germinated to the extent of four percent in the same length of time (table 3 and text fig. 4).

Production of seedlings was much more prompt and complete than that reported by Toumey and Stevens (6). They found that the earliest germination was in 50 days and that in most samples there was no germination within the period of the test. The average germination in 50 days was reported as 0.7 percent and the highest three percent.

*Thuya gigantea*, *Thuya occidentalis*, and *Thuya orientalis* all responded to two months' stratification at 5° C. In each case the treatment resulted in more prompt and complete stands of seedlings than in the control lots of seeds although this difference was more marked in the case of *Thuya gigantea* (table 3 and text fig. 1, left).

The results agree in general with the germination of *Thuya occidentalis* as reported by Toumey and Stevens (6). They found that the highest germination in 50 days was 65 percent while the average was 33.9 percent. In their samples germination started from ten to 15 days after beginning the test and the crest was usually reached within a period of 25 days.

#### SUMMARY

1. Experiments on the seeds of several coniferous trees revealed a general favorable effect of low temperature stratification on seed germination.

2. Sample plantings made in the greenhouse after stratification in moist acid peat at 5° C. for a period of two months not only saves time in seedling production but also produces more complete seedling stands in the majority of cases. In some instances other stratification temperatures or periods or both prove more advantageous.

3. *Pinus austriaca*, *Pinus Banksiana*, *Pinus Laricio*, and *Pinus ponderosa* gave a much more prompt production of seedlings (stand complete 8 to 48 days sooner) after stratification for two months at 5° C. However, the actual number of seedlings produced from treated and untreated seeds was about the same.

4. *Pinus contorta*, *Pinus contorta Murrayana*, *Pinus Coulteri*, *Pinus densiflora*, *Pinus flexilis*, *Pinus insignis*, *Pinus monticola*, *Pinus resinosa*, *Pinus rigida*, *Pinus Strobus*, *Pinus Thunbergii*, *Abies arizonica*, *Libocedrus decurrens*, *Picea Omorika*, *Picea sitchensis*, *Thuya gigantea*, *Thuya occidentalis*, and *Thuya orientalis* show decided beneficial effects of stratification at 5° C. for a period of two months. Not only are the seedlings produced in a shorter period of time, but the actual number of seedlings produced is greater than in the corresponding controls.

5. Three months' stratification at 10° C. resulted in the best germination (8 percent after 25 days) of *Pinus excelsa* obtained in these tests. This was better than the average control (2 percent in 60 days), but further study is needed.

6. *Pinus koraiensis* responded equally well to stratification for three months at 5° C. or for one or two months at 10° C. However, the best germination (33 percent in 43 days) was obtained after the seeds had been stratified for five months at either 0° or 5° C.

7. *Pinus Lambertiana* seeds gave best germination after stratification for three months at 10° C. *Pinus Lambertiana* II 1928 thus treated yielded 92 percent germination in 25 days while the average control of the same lot had produced no seedlings in 70 days.



TABLE 2.—Continued

Species	Months of Stratification	0° C.			5° C.			10° C.			Average Control		
		Appar-ent % Germ.	Real % Germ.	Days	Appar-ent % Germ.	Real % Germ.	Days	Appar-ent % Germ.	Real % Germ.	Days	Appar-ent % Germ.	Real % Germ.	Days
<i>Pinus contorta</i> , 1928.	1	69	70	14	77	78	11	57	58	45	47	47	18
	2	67	68	18	86	89	18	65	66	23	—	—	—
	3	64	65	12	84	85	15	—	—	—	—	—	—
<i>Pinus contorta Murrayana</i> , 1927.	1	1	1	15	2	2	8	—	—	—	4	4	16
	2	2	2	11	6	6	—	—	—	—	—	—	—
	3	0	0	50	0	0	50	6	6	30	—	—	—
<i>Pinus contorta Murrayana</i> , I, 1928.	1	80	82	13	83	85	13	71	72	45	63	64	24
	2	77	79	14	90	92	14	63	64	23	—	—	—
	3	72	73	9	87	89	12	53	53	20	—	—	—
<i>Pinus contorta Murrayana</i> , II, 1928.	1	70	71	14	81	82	14	31	31	45	40	40	20
	2	59	60	14	64	65	11	27	27	27	—	—	—
	3	56	57	12	72	73	9	44	44	15	—	—	—
<i>Pinus contorta Murrayana</i> , III, 1928.	1	75	77	26	77	79	26	59	60	32	28	29	40
	2	79	81	18	78	80	18	18	18	27	—	—	—
	3	82	84	9	94	96	15	18	18	25	—	—	—
<i>Pinus Coulteri</i> , 1928.	1	80	83	24	90	94	19	70	73	24	0	0	70
	2	100	104	19	100	104	19	100	104	19	—	—	—
	3	100	104	25	88	92	25	100	104	25	—	—	—
<i>Pinus densiflora</i> , 1927.	1	57	59	31	68	71	25	—	—	—	38	40	40
	2	76	79	21	70	73	24	—	—	—	—	—	—
	3	36	38	30	68	71	30	82	87	23	45	48	28
<i>Pinus densiflora</i> , 1928.	1	63	67	23	60	64	23	—	—	—	—	—	—
	2	66	70	23	52	55	23	—	—	—	—	—	—
	3	57	61	12	67	71	20	—	—	—	—	—	—
<i>Pinus excelsa</i> , 1927.	1	0	0	60	0	0	60	2	3	28	2	3	60
	2	0	0	60	0	0	60	2	3	21	2	3	—
	3	0	0	60	1	1	37	2	3	25	—	—	—
<i>Pinus excelsa</i> , 1928.	1	2	3	34	1	1	18	0	0	45	1	1	12
	2	0	0	49	1	1	27	8	11	14	—	—	—
	3	0	0	53	2	3	20	1	1	25	—	—	—
<i>Pinus flexilis</i> , 1927.	1	86	104	17	94	113	17	42	51	22	46	55	28
	2	86	104	14	100	120	18	80	96	23	—	—	—
	3	96	116	12	88	106	9	96	116	15	—	—	—
<i>Pinus flexilis</i> , 1928.	1	45	83	11	35	65	14	55	102	23	45	83	28
	2	18	33	23	40	74	23	32	59	23	—	—	—
	3	36	67	15	36	67	15	48	89	25	—	—	—

TABLE 2.—Continued

Species	Months of Stratification	0° C.			5° C.			10° C.			Average Control		
		Appar-ent % Germ.	Real % Germ.	Days	Appar-ent % Germ.	Real % Germ.	Days	Appar-ent % Germ.	Real % Germ.	Days	Appar-ent % Germ.	Real % Germ.	Days
<i>Pinus insignis</i> , 1927.	1	69	71	33	81	84	18	28	29	15	62	64	50
	2	75	78	24	75	78	14	—	—	—	—	—	—
	3	62	64	30	84	87	30	86	89	25	—	—	—
<i>Pinus insignis</i> , 1928.	1	74	80	28	77	83	18	85	91	28	65	70	28
	2	62	67	14	79	85	18	76	82	18	—	—	—
	3	60	65	15	84	90	20	90	97	15	—	—	—
<i>Pinus koraiensis</i> , I, 1928.	1	0	—	60	15	—	45	—	—	—	—	—	—
	2	8	—	64	8	—	27	8	—	82	0	0	70
	3	0	—	53	20	—	25	0	—	53	—	—	—
<i>Pinus koraiensis</i> , II, 1928.	2	0	0	47	0	0	47	10	10	28	—	—	—
	3	0	0	47	0	0	47	10	10	28	0	0	70
<i>Pinus Lambertiana</i> , 1927.	1	10	10	25	10	10	25	0	0	70	—	—	—
	2	14	14	62	33	33	62	9	9	55	2	2	70
	3	72	72	30	67	67	37	47	47	37	—	—	—
<i>Pinus Lambertiana</i> , I, 1928.	1	0	0	60	0	0	60	0	0	60	—	—	—
	2	28	32	64	32	36	64	8	9	55	8	9	70
	3	30	34	20	50	57	35	30	34	25	—	—	—
<i>Pinus Lambertiana</i> , II, 1928.	2	80	103	19	40	51	22	28	36	22	—	—	—
	3	72	92	25	64	85	25	92	118	25	0	0	70
<i>Pinus Laricio</i> , 1927.	1	42	48	15	59	66	15	—	—	—	—	—	—
	2	48	55	14	50	57	14	—	—	—	—	—	—
	3	34	39	22	33	38	25	—	—	—	56	64	26
<i>Pinus Laricio</i> , 1928.	1	32	32	13	21	21	7	—	—	—	—	—	—
	2	7	7	12	13	13	6	6	6	13	—	—	—
<i>Pinus monicicola</i> , 1927.	1	20	25	33	10	13	28	1	1	12	11	11	26
	2	37	47	62	26	34	62	23	29	28	12	15	60
	3	24	30	37	41	54	37	22	28	50	—	—	—
<i>Pinus monicicola</i> , 1928.	2	28	29	47	15	16	21	7	7	47	—	—	—
	3	30	32	23	18	19	16	17	18	28	10	11	50
<i>Pinus ponderosa</i> , I, 1927.	1	74	85	15	76	87	10	78	90	15	—	—	—
	2	78	90	14	70	80	17	68	78	14	73	84	22
	3	—	—	—	63	72	37	—	—	—	—	—	—
<i>Pinus ponderosa</i> , II, 1927.	1	97	103	28	97	103	28	88	94	31	79	84	40
	2	60	64	22	71	76	14	46	49	22	—	—	—

TABLE 2.—Continued

Species	Months of Stratification	0° C.			5° C.			10° C.			Average Control		
		Appar-ent % Germ.	Real % Germ.	Days	Appar-ent % Germ.	Real % Germ.	Days	Appar-ent % Germ.	Real % Germ.	Days	Appar-ent % Germ.	Real % Germ.	Days
<i>Pinus ponderosa</i> , 1928.....	1	47	55	14	36	42	14	62	73	18	48	56	16
	2	30	38	18	47	55	14	39	40	11			
	3	85	18	12	48	86	18	84	87	15			
<i>Pinus resinosa</i> , 1927.....	1	88	91	18	83	86	18	84	87	15			
	2	73	75	14	91	94	17	83	86	17	64	66	24
	3	76	78	22	95	98	22	89	92	25			
<i>Pinus resinosa</i> , 1928.....	1	89	94	18	84	88	18	91	96	18	67	72	24
	2	—	—	—	89	94	18	97	102	23			
	3	90	95	15	85	89	15	79	83	15			
<i>Pinus rigida</i> , 1928.....	1	86	95	18	95	104	18	79	87	23			
	2	79	87	14	92	101	14	69	105	14	32	35	40
	3	83	91	12	88	97	12	85	93	15			
<i>Pinus Strobus</i> , 1927.....	1	57	59	33	47	49	31	59	61	31	3	3	60
	2	70	73	41	71	74	41	76	79	41			
	3	56	58	37	64	67	37	52	54	40			
<i>Pinus Strobus</i> , 1928.....	1	27	28	45	26	27	28	16	16	45	6	6	40
	2	35	36	23	26	27	23	46	47	23			
	3	28	29	35	33	34	25	16	16	25			
<i>Pinus Thunbergii</i> , 1927.....	1	12	14	25	16	19	25	15	18	15	8	9	30
	2	10	12	21	20	24	21	8	9	14			
	3	7	8	40	6	7	37	6	7	25			
<i>Pinus Thunbergii</i> , 1928.....	1	14	18	23	18	23	28	7	9	18			
	2	7	9	23	11	14	23	12	15	23	10	13	28
	3	10	13	25	5	6	12	6	8	15			
<i>Sciadopitys verticillata</i> , 1928.....	2	42	46	148	42	49	117	50	56	101			
	3	42	49	117	42	49	117	36	40	117	28	31	100
<i>Taxodium distichum</i> , 1928.....	1	19	25	28	39	51	28	40	53	28	4	5	24
	2	32	42	37	31	41	27	50	66	27			
	3	43	57	26	27	36	16	33	43	23			
<i>Thuja gigantea</i> , 1928.....	1	72	90	16	67	84	16	56	70	19			
	2	43	54	12	57	71	14	43	54	14			
	3	60	75	14	74	93	14	56	70	14	10	13	24
<i>Thuja occidentalis</i> , 1928.....	1	28	51	18	50	91	24	41	75	30			
	2	40	73	20	33	60	24	44	80	20	24	44	24
	3	38	69	16	22	40	16	40	73	16			
<i>Thuja orientalis</i> , 1928.....	1	20	27	18	21	28	18	26	35	18			
	2	33	45	27	33	45	18	9	12	12	26	35	24
	3	36	49	12	30	41	12	—	—	—			

TABLE 3. *Apparent Germination Percentages after Stratification at 5° C. for One, Two, and Three Months. Percentages Given at Two-day Intervals up to 30th Day, after Which the Intervals Are Ten Days. Germination Values Are for 100 Seeds Unless Otherwise Indicated. Controls Are Averages of Separate Controls for One, Two, and Three Month Stratification Periods*

[illegible]







TABLE 3.—Continued

[illegible]





## IS THE ASTER-YELLOWS VIRUS DETECTABLE IN ITS INSECT VECTOR?

IRENE D. DOBROSCKY

The leaf hopper, *Cicadula sexnotata* Fall., has been shown to be the specific carrier of the virus of aster yellows (6). Furthermore, this virus must remain in the body of the insect for at least ten days before it can reinfect another plant. Similar conditions have been found to obtain in the case of curly top of sugar beets and the leaf hopper, *Eutettix tenellus* (Baker) (8); and, also, in the case of streak of maize and the leaf hopper, *Balclutha mbila* Naude (9). The necessity for an incubation period of the virus would seem to indicate that we are dealing with an organism which must either multiply in the insect or undergo a definite part of its life cycle there. This simulates closely the facts known about malaria, yellow fever, Texas cattle fever, nagana disease of cattle, and African sleeping sickness.

Because of the long incubation period of the aster-yellows virus in its insect vector it was thought this leaf hopper might be a favorable one in which to detect the virus. An intensive study of the morphology and cytology of the insect was therefore undertaken. Such a study might result in the discovery of the causative agent of yellows or some evident reaction of the insect to the virus. It might result even in the discovery of an intracellular body associated with the virus in the insect.

Botanists who have studied the tissues of plants affected with virus diseases have found intracellular bodies in connection with many of these diseases. At least twenty-eight species of plants having the mosaic type of virus disease have been reported as containing these so-called "x-bodies," or inclusion bodies.

Plants affected with the yellows type of virus diseases, such as aster yellows, peach yellows, curly top of sugar beets, and cranberry false blossom, have not been found to contain intracellular bodies. The morphological symptoms of a plant affected with aster yellows are, however, very striking and characteristic. Nevertheless, no bacterium, protozoan, or fungus has been found associated with the disease. The causative agent has not been found in those plants susceptible to the aster-yellows virus. It seemed advisable to search for it in the insect.

### METHODS

The histology and cytology of the leaf hopper, *Cicadula sexnotata*, were studied by making smears and examining these under dark-field illumination, by gross dissections of the alimentary tract, and by serial paraffin sections.

Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.

*Smears.* In making smears about fifty adults, hatched and reared on diseased asters, were used. Similar virus-free adults served as checks. Each insect was slowly crushed between a slide and a cover slip and, while the smear was still wet, it was examined under dark-field illumination. Numerous globules of fat were observed, some showing Brownian movements. There was no indication of vital movement shown by any of the bodies in the smears.

When the smears had dried they were stained with Wright's blood stain for one minute. All the slides showed small brownish granules which were the pigment granules of the compound eyes. About one-half of the smears contained oval-shaped bodies  $8\ \mu$  to  $12\ \mu$  long, with pink capsule-like rims, blue cytoplasm, and bright red nuclei. A few forms were found dividing. These bodies resemble the spores of *Cladosporium*, a soil-infesting fungus. As the spores were never found in the paraffin sections, it is probable that the insects bore them externally. Nuclei of all sizes and stages of activity were found in the smears. Crushed nuclei in the spireme stage took a pink stain and resembled clumps of bacteria. Many fragments of broken sperms were found but there was nothing present resembling either a bacterium or a flagellate.

*Dissections.* Gross dissections were made to study the alimentary canal. The stomach was found distended with a dark brown fluid. Continuing from the stomach is a narrower portion of the midintestine. This is very white and opaque in appearance, due to the accumulation of calcium-carbonate crystals within the cells. It is thought that the liquid food passes by osmosis from the stomach to the posterior intestine without passing through this narrow portion of the midintestine. Two of the most important appendages of the alimentary canal are the salivary glands and Malpighian tubules. The Malpighian tubules are four in number, rather large and thick. The salivary glands are in the head and prothorax. They are so small and so transparent that it is difficult to find them.

No cysts, lesions, or abnormal enlargements of any organ were found in the course of this macrodissection.

*Paraffin Sections.* More than a thousand insects, adults, and nymphs were fixed in various reagents, including Gilson's, Regand's, Bouin's, Flemming's weak, and Carnoy's solution. The sections were cut at  $4\ \mu$  or  $7.5\ \mu$ . They were then stained with Wright's, Giemsa's, Wolbach's Modified Giemsa, Heidenhain's Haematoxylin, Flemming's, Mallory's Methylene blue, Pianese III B, Gentian violet, and Safranin, or acid fuchsin stain. For secretory cells of the salivary-gland type, Gilson's fixative followed by Wright's Romanowsky stain gave the best results. The Romanowsky stain, as put up by Coleman and Bell Co., proved most satisfactory. McNeal (7) has recently shown that there are four essential dyes in this stain. They are eosin, methylene blue, methylene azure, and methylene violet.

## OBSERVATIONS

The parasites which cause malaria are found in cysts in the stomach walls and also as free swimming flagellates in the salivary glands of their mosquito hosts. *Herpetomonas* associated with oriental sore was found in the salivary glands of the fly *Phlebotomus papatasi* (1). Holmes (5) found *Herpetomonas elmassiani* (Migone) in the salivary glands of *Onopeltus fasciatus* Dall., a bug which feeds on milkweeds. The flagellate, *Leptomonas davidii* Lafont, was found by França (4) in the salivary glands of the hemipteron *Stenocephalus agilis* Scop. which feeds on Euphorbias. These organisms are all ingested by their insect hosts. In several insect-borne diseases the causative organism has been found in the alimentary tract, proper. It seemed advisable, therefore, to make an intensive study of the salivary glands and the alimentary tract of the leaf hopper in question in the hope of finding some indication of the presence of the virus.

*Salivary glands.*<sup>1</sup> The salivary glands lie beneath the brain and occupy the dorsal floor of the head. They are made up of equal halves lying on either side of the œsophagus. Each half resembles a small bunch of grapes with a fore-shortened central stem. The lobules, twenty-three in each half, are large secreting cells containing two nuclei. Physiologically, the salivary glands may be divided into three types of cells, the mucous cells, the serous cells, and the cells which line the accessory or reservoir glands.

In making a comparative study of the salivary glands of healthy and viruliferous individuals, the following characteristics were noted: 1. Size, shape and staining properties of each lobule; 2. Texture and staining reaction of cytoplasm; 3. Vacuoles and inclusions in cytoplasm; 4. Size, staining properties and physiological state of nuclei; 5. Amount of secretion.

Though several hundred insects were examined with these points in mind, no constant difference was observed. No lesions, no cysts, no difference in size or shape of lobules, nor any alteration in the physiology of the cells, as indicated by their staining reactions, could be found.

After this study of the cytology of the secreting cells, the author proceeded to look for any foreign organism that might be present. The numerous stains used were calculated to detect the presence of bacteria, fungi, protozoa, Rickettsia or x-bodies. None of these was found in the salivary glands of either the normal or the viruliferous individuals.

In one experiment a series of adults, fed from one to twenty-three days on diseased asters, was studied in the hope of finding some progressive symptoms to account for the incubation period of the virus. No symptom indicative of the presence of the virus could be found.

<sup>1</sup> A more detailed morphological description of the salivary glands will be published by the author in another paper.

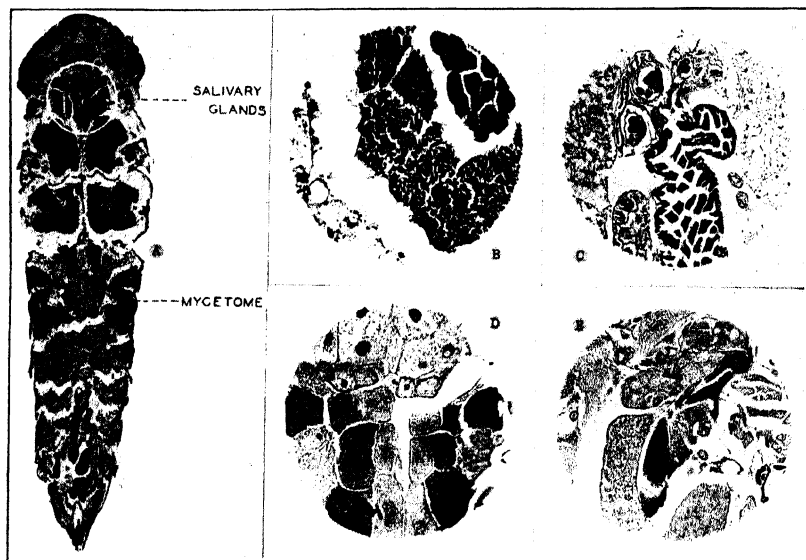


FIG. 1. A. Longitudinal section of adult female of *Cicadula sexnotata*. Shows salivary glands in prothorax and mycetome on either side of the first and second abdominal segments. ( $7\ \mu$  thick,  $\times 50$ .)  
 B. Mycetome in detail. Note zone of empty cells with small marginal nuclei. ( $7.5\ \mu$ , thick  $\times 430$ .)  
 C. Midintestine of disease-carrying insect. ( $7\ \mu$  thick,  $\times 180$ .)  
 D. Salivary glands of adult insect starved ten hours. ( $7\ \mu$  thick,  $\times 200$ .)  
 E. Lateral view of salivary glands of healthy normal insect. ( $7\ \mu$  thick,  $\times 160$ .)

In another experiment some adults were starved and their salivary glands compared with those of normal insects in order to see how the cells were altered. It was found that the individual cells of the glands of starved insects were larger, due to an accumulation of secretion. This is illustrated in figure 1, D. Figure 1, E, shows the salivary glands of a normal healthy insect.

*Alimentary tract.* It is a well known fact that most insects shed their chitinous covering several times during the course of their development. The anterior and posterior intestines are lined with a thin intima, a continuation of the outer heavy chitin. This intima is shed along with the outer coat when the insect molts. An insect which has fed on a diseased plant and becomes infective does not lose its infective principle in spite of this process of shedding. Since the midintestine is not lined with intima, it would seem that this is the logical place to look for obligate parasites. Figure 1, C, is a photograph of the anterior portion of the midintestine, showing the large darkly stained cells which compose it.

It has been found in the case of several insect-borne diseases that the parasites are first localized in some part of the alimentary canal and from there get into the salivary glands. *Cicadula sexnotata* was found remarkably free from all parasites. Its alimentary tract does not harbor any foreign organisms.

Cowdry (3) examined one hundred and eleven species of insects and found that nineteen contained Rickettsia bodies. The writer, using Cowdry's methods of staining and fixing was unable to find any Rickettsia in the body of this leafhopper.<sup>2</sup>

*Mycetome.* The mycetome is an organ present in most Homoptera. This might easily be mistaken for a sign of the disease in the insect under discussion. Witzlil (11) regarded it as excretory in function, others ascribed to it a nutritive function. Sulz (10), Buchner (2), and Glaser, however, have concluded that the organ is parasitic.

The mycetome is a large paired organ located in the first two abdominal segments, as shown in figure 1, A. The cells composing it are closely packed with organisms of a fungal nature, whence the organ derives its name.

Three distinct zones, comprising different types of cells, can be seen in this organ. Figure 1, B, an enlargement of a portion of the mycetome, shows these zones very clearly. In the center of the organ are numerous compact cuboidal cells. The nuclei are almost one-half as large as the cell, and the cytoplasm is densely filled with a mycelium-like growth. With Wright's stain this zone is colored dark blue. The next zone is composed of very much larger cells with smaller nuclei. The cytoplasm is obscured by the presence of the thick mycelium-like strands. This zone stains a pinkish purple. The third zone is one-cell thick, the cells being almost empty of cytoplasm. Nothing but small, compact, dark blue nuclei are to be found along the borders of these outer cells.

The whole organ is embedded in the fat tissue and has no direct connection with any other organ. It is present in virus-free as well as virus-bearing individuals. The organisms within this mycetome are supposed to migrate to the eggs and are so transmitted from one generation to another. Since this organ is hereditary and since it is not in communication with any other organ, it is not likely that it can be concerned in the problem of the insect's ability to harbor the yellows virus.

#### DISCUSSION

The virus diseases of plants are essentially insect-borne. Sucking insects, especially aphids and leaf hoppers, are the chief disseminating agents

<sup>2</sup> Dr. Cowdry was kind enough to examine some of the writer's slides and confirmed this conclusion.

under natural conditions. Of the leaf hoppers known to be virus vectors, all but one are very closely related in the classification of insects.

Up to the present time, no one has done any intensive research on the morphology and cytology of these insect vectors with the view to locating the virus. The writer has been unable to find any histological or cytological differences between virus-free and virus-bearing individuals of the species *Cicadula sexnotata*. While the technique used has given only negative data, it is possible that other methods of fixing and staining may yield positive results.

A study of the other insect carriers of plant viruses may give a clue as to where the yellows virus may be located in the body of the insect. Physiological studies may also throw light on the subject. Significant results might be obtained by making determinations of the pH of individual insects. If certain portions of virus-carrying individuals were isolated and healthy insects made to feed on them, one might get an indication of the whereabouts of the virus. Several attempts have been made to culture the virus in plant decoctions. Some experiments in culturing the virus in insect juices should also be undertaken.

#### SUMMARY

A study of smears of virus-bearing insects under dark field illumination, of gross dissections, and of paraffin sections did not reveal any visual evidence of the presence of the aster-yellows virus in *Cicadula sexnotata*.

The mycetome, a hereditary parasitic organ, is evidently not concerned in the problem of the virus-carrying ability of the insect.

After an intensive study of the salivary glands and alimentary tract of *Cicadula sexnotata* with a view to finding the causative agent of aster yellows, the writer was unable to find such an agent or any lesions which might be due to the presence of such an agent. No bacterium, Rickettsia, fungus, protozoan, or x-body was found which could be considered of etiological significance.

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## INFLUENCE OF ENVIRONMENT ON THE CALLUSING OF APPLE CUTTINGS AND GRAFTS

WILLIAM B. SHIPPY

### INTRODUCTION

A particular impetus for the study of callus physiology has been given by the results of recent investigations with apple crown gall which tend to show that the larger proportion of overgrowths on root-grafted trees are not bacterial tumors but callus tumors, formed chiefly as proliferations of the scion lip (Melhus, 1926). The fact that overgrowths may be non-parasitic in origin does not minimize their importance, but throws the emphasis of control on the regulation of growth processes of individual plants. This would obviate the consideration of their spread from one plant to another, the problem being rather one of inhibiting the excessive formation of callus cells at the graft union because of the frequent occurrence of overgrowths at this point.

The literature reveals many references to callusing, particularly with respect to the derivation and growth of callus cells and tissues. Of particular importance in this connection are the studies of Trécul (1853), Göppert (1874), Stoll (1874), Küster (1903), and Sorauer (1908). Küster and Sorauer have brought together most of the general information with respect to callus available up to 1900.

Though environmental factors have been studied many times in their relation to plant growth processes in general, the particular influence of these factors on the formation of callus tissue has received scant attention. Küster (1903) pointed out that external conditions influence callusing, and of these moisture is very important. He stated that moisture, supplied either in gaseous or in liquid state, is essential for callus development, and further, that although callus may form under water, its formation is much more abundant in moist air, supposedly because transpiration and the absorption of oxygen are hindered under water. Nutritive conditions were considered by Küster to influence callusing. Organs rich in elaborated food were believed to develop wound tissue more abundantly than those poor in such materials. Under similar external conditions the capacity of both cut ends of a cutting for forming callus was not found to be equal. With poplar cuttings the basal poles callused more abundantly than the apical. Similar results were obtained with *Rosa*. With dandelion roots, callus formed more readily from the upper end, but with root cuttings of *Medicago sativa* more callus developed from the lower or root end. Küster

was unable to decide what factors influenced this polarity, but considered inequalities in nutritive conditions important.

Simon (1908) attempted to control temperature and moisture in his study of callus formation on cuttings of *Populus nigra* and *Populus canadensis*. He found that at 14°–18° C. callus formed after eight days, at 25° after four days, and at 32° after three days. The apical and basal ends of cuttings were found to respond differently to humidity. Whereas apical callusing was greatest at 85 to 90 percent relative humidity, basal callusing was best at humidities between 90 and 94 percent. At complete saturation callusing became more or less equalized after a time for both ends of the cuttings, although at first callus formed more abundantly from the basal end.

Riker and Keitt (1926), in discussing the development of excess callus and wound overgrowth in the case of apple root-grafts, stated that "The influences of temperature and, even more particularly, of moisture are of very great importance in determining the extent of callus development." These authors report studying the influence of temperature and moisture on the development of callus on apple grafts but present no data on this work. They found that "within certain limits callus development increased with higher temperatures and moistures and decreased with lower temperatures and moistures." A relation of food to overgrowth formation is suggested by the statement: "Injuries such as those from cultivators, hoes, hooks, and insects may serve to stop the downward passage of elaborated food and to lead to developments similar to those which follow a poor fit in grafting. Such developments are normal processes of the plants when suitable conditions are provided." And further, in referring to the excessive formation of callus from the scion lip, they say, "It seems probable that the food as it descended the twig to the cut made a slow lateral movement along an obtuse angle to the lower tip where it accumulated and contributed to the development of callus. In a well-fitted graft such an accumulation occurred in a much smaller degree because the food material passed through the united cambium layers into the root." They report that "wound overgrowths have been observed to reach considerable size on some plants and to continue their development for several years."

Rehwald (1927) found that callus formation from root cuttings of *Daucus Carota* was facilitated by water-saturated air. He also found that the top and base of such cuttings differed in their capacity for callus formation, obtaining callus only from the basal portion of the cutting.

Swingle (1929) studied the relation of temperature, water, and oxygen to apple root growth and callus development. He used chiefly stem cuttings of the apple variety Springdale and the willow (*Salix alba* L.). The apple cuttings were all obtained from a single tree, representing wood from three to ten years old. Callusing was usually determined for a ten-day period only. In these experiments temperature appears to have been

well controlled. Moisture was supplied in two ways: either the cuttings were held in a saturated atmosphere, or during the callusing period or previously they were partly or entirely immersed in water. In the study of aëration, oxygen was supplied in three concentrations: 5 percent, 20 percent (air), and pure oxygen, these gas mixtures being passed through the control chambers at different rates of flow. Swingle concluded that a preliminary treatment of the plant material with water retarded callusing. Callusing seemed to be more active with slightly higher temperatures and with somewhat lower water and oxygen supplies than were indicated for the most active production of roots. Under good environmental conditions he found callus formation to be more active at the basal end of the cuttings, suggesting an internal polarity. Oxygen (100 percent) at a pressure of one atmosphere distinctly retarded callus formation.

Hitchcock (1928), in studying rooting response, found that good callus formation occurred on many hard-wood cuttings in a peat moss medium of low moisture content (140 percent).

Kostoff (1928) studied the graft unions of intergeneric and interspecific crosses between members of the Solanaceae. He came to the conclusion that the callus tissues joining scion and stock are chiefly the product of the stock. Various types of tumors superficially like crown gall were observed immediately above the callus. Microscopical examination showed that large quantities of starch, produced by the scions, had accumulated just above the callus due to the fact that the union intercepted their passage. This large accumulation of food was considered the specific cause of the proliferations.

Miss Smith (1928), working with *Clematis*, found that the "amount of callus formed by stem cuttings varies with the age of the wood taken, the amount of food reserves, as well as with the anatomical structure of the species," but that "other factors no doubt come into play." She states further that "There is an undoubted correlation between the amount of starch present in the tissues and the amount of callus formed by any given cutting, though it is not yet possible to express it as a quantitative relation." Different species of *Clematis* were found to vary in degree of callus formation. Under similar temperature conditions (bottom heat of 18.3° C.) *C. afoliata*, *C. smilacifolia*, and *C. uncinata* produced callus slowly and the total amount formed was small, while such species as *C. Armandi*, *C. Hillarii*, and *C. ranunculoides* produced large calluses at a comparatively rapid rate. She pointed out that following the cutting injury to a stem there are death-changes in the divided protoplasts due to the altered metabolism of the injured cells. Following these death-changes a suberin seal is laid down, and still later the first signs of abnormal cell divisions may be seen. These processes were described for *C. smilacifolia*.

The present study was undertaken for the purpose of obtaining quantitative information regarding the influence of such primary environmental

factors as temperature and moisture on the callusing of apple root-grafts. The broad application of such information to horticultural practices is obvious. It was further considered possible that a greater knowledge of environmental influences on callusing might throw light on the problem of how these conditions may be manipulated to favor or restrict excessive callus formation, and that detailed observations on the callusing of scion and stock under many varied conditions might contribute toward a better understanding of the factors influencing the production of overgrowths.

### MATERIALS AND METHODS

The study was begun at the Boyce Thompson Institute for Plant Research during the spring of 1927 and was continued for two years. The early part of the work was devoted to a preliminary survey of the comparative importance of many different environmental factors on callusing in addition to a search for satisfactory methods of obtaining quantitative information on the effect of certain conditions. A general discussion of the materials used and the methods followed is presented herewith, and detailed procedures are given later for each individual experiment.

*Plant Material.*—With only a few exceptions, one-year-old shoots and one-year-old seedling root-stocks were employed. The scion material consisted of standard straight whips grown in a scion orchard, and represented the following varieties: Jonathan, Wealthy, Yellow Transparent, Ben Davis, Grimes, Delicious, Wolf River, Willow Twig, Northwestern, and Winesap. Scions were obtained chiefly from the Mount Arbor Nursery, Shenandoah, Iowa, though some were secured from the Chase Brothers Company, Rochester, New York. Occasionally material of unknown variety was used; this was obtained from trees located on the premises of the Boyce Thompson Institute, Yonkers, New York.

A number of kinds of root-stock material were used, including French crab, Kansas grown; French crab, French grown; Austrian crab, California grown; seedlings of northern hardy varieties, Minnesota grown; and Tennessee crab, Kansas grown. Later the following were added to the list: Vermont crab, Kansas grown; seedlings of northwestern varieties, Washington grown, as well as the Hopa and Meador's winter flowering crabs, Kansas grown. These stocks were obtained from J. H. Skinner and Company, Topeka, Kansas; Vistica Nurseries, Inc., Stockton, California; Oliver Nursery Company, Topeka, Kansas; Clinton Falls Nursery Company, Owatonna, Minnesota; Washington Nursery Company, Toppenish, Washington; and Chase Brothers Company, Rochester, New York.

*Preparation of Plant Material.*—The scion and root-stock material was callused both as grafts in which the two symbionts were combined (Pl. XXII, figs. 1 and 2) and as cuttings in which they were separate (Pl. XXIII, fig. 6). In either case the individual pieces were cut as they would be in making a tongue graft; that is, a diagonal cut about 3 centimeters ( $1\frac{1}{4}$  inches)

in length was made on the bottom end of the scion cutting, and a similar cut was made on the top end of the root cuttings (bottom in this sense meaning downward and top upward with respect to the ground line.) All cuts were carefully made with a sharp grafting knife. Grafts were not used entirely because of the great difficulty of measuring the callus of a graft union. In those experiments in which grafts were used, a sufficient number of individuals was taken so that after breaking the union apart and examining the callus formed, the graft could be discarded. Many tests have indicated that the influence of the environment on callus formation at the union of a graft could be determined just as accurately with the scion and stock separate as with them together. In some of the later experiments both ends of the cuttings were cut off squarely with hand shears, and then the roughly cut surfaces were trimmed with a sharp knife so as to provide a smooth, even surface. It is less difficult to measure the amount of callus in a symmetrical roll than in an asymmetrical roll such as is formed with the slanting cut where each area along the slant may differ in its ability to form callus.

The cuttings usually averaged from 12 to 15 centimeters (5 to 6 inches) in length, scion cuttings usually being longer than stock cuttings. The scion cuttings were cut apically immediately above a bud; at the slanting-cut end a bud was left on the back of the lip, since this is the custom among horticulturalists. Tongue grafts were usually employed for the callusing experiments, although wedge grafts and modifications of both tongue and wedge grafts were used from time to time. The procedure for making a tongue graft is so well known that it need not be repeated here.

The plant material was never allowed to dry. Before being cut up for the experiments, the shoots and root-stocks were stored in moist peat moss at a temperature of about 3° C. In this way the plant material has been kept in satisfactory condition for nearly two years. After cutting, special care was taken to prevent the cut surfaces from becoming injured by drying. In some instances when the cuttings were to be used immediately, they were dropped into a pan of water, but the more common procedure was to place them in moist peat moss until the experiment was begun. Experiments requiring only a small amount of plant material were usually begun the same day that the material was cut. For larger experiments, requiring from several hundred to several thousand cuttings or grafts, the material was placed in moist peat moss at 3° C. as soon as prepared until all pieces could be started simultaneously.

*Temperature Experiments.*—For the study of the influence of temperature several types of equipment were used, including (1) electrically-heated ovens, (2) refrigeration rooms, (3) temperature-controlled greenhouses, (4) basement storage room, (5) outdoor storage cellar, and (6) cold frame. Each type has its advantages as well as its limitations, but the combined conditions that were available permitted the study of constant and variable temperatures, of slowly and sharply fluctuating temperatures, and allowed

the use of small or large amounts of plant material, together with such apparatus as was required.

The controlled ovens ranged in temperature from 0° to 40° C., with a separate oven for each 5° between these two extremes. The refrigeration rooms were operated at 3°, 10°, 15°, and 20° C. Temperatures in the greenhouses showed considerable fluctuation over the 24-hour period, but the automatic steam control prevented a drop in temperature below any specified point. Both the basement storage room and the outdoor storage cellar maintained a fairly even temperature, but both were subject to the influence of any prolonged change in outdoor temperatures. The temperature of the cold frame varied almost in accordance with that outside.

*Moisture Experiments.*—To procure and maintain a graded series of moisture conditions was not easily accomplished. Several methods were tried, no one of which was wholly satisfactory. The control of relative humidity was attempted chiefly in two ways: by means of sulfuric acid solutions, and by means of saturated solutions of inorganic salts. Each of these methods presents difficulties. The sulfuric acid solution becomes more and more dilute as it takes up water from the surrounding plant material. As a result the concentration and corresponding vapor pressure change. Additional acid must be added to restore the original concentration. The saturated salt solution has the advantage that it is self-regulatory as to vapor pressure. As water vapor is taken up by the solution the undissolved crystals go into solution, serving to keep the concentration uniform.

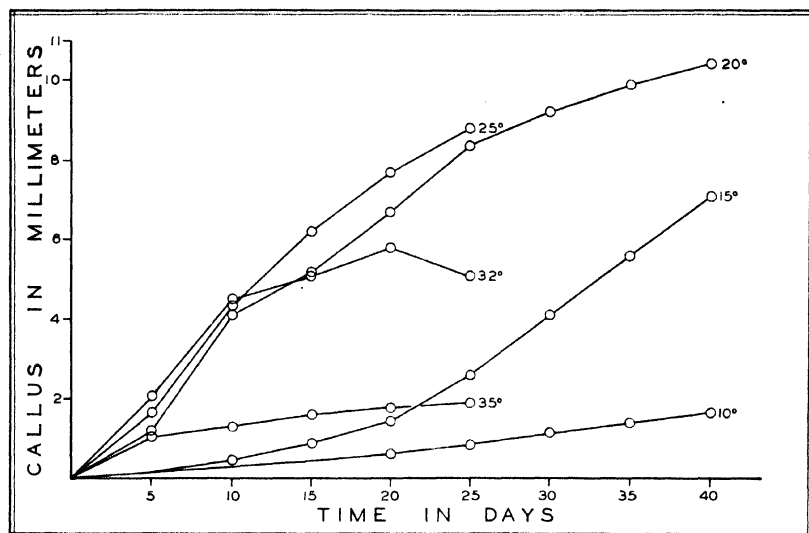
Open systems (in which fresh air was constantly supplied) and closed systems (using stoppered chambers) were used. These systems were operated at various temperatures. With each system sulfuric acid solutions and inorganic salt solutions were used to control vapor pressure. Controlled chambers used in the various tests consisted of test tubes, bottles, desiccators, and crocks up to 12 gallons in capacity. Further, the air in the chambers was circulated by motor-driven fans in some experiments and not in others.

For studies of the influence of relatively high supplies of water, the plant materials were placed in direct contact with peat moss having various water contents. Here little difficulty was encountered. Representative samples of the peat moss were taken from time to time, and the water content was determined by weight. The lack of complete uniformity of moisture in the peat moss and the necessity for taking representative samples comprised the main difficulties; these were, however, not very serious. For these experiments use was made of both covered and uncovered containers, including test tubes of various sizes, desiccators, crocks, and greenhouse flats.

*Aëration Experiments.*—To study the influence of oxygen on the formation of callus a number of experiments were carried out in which various

mixtures of oxygen with air or nitrogen were used. The oxygen content of the mixtures ranged from 0 to 100 percent. In these experiments a fresh supply of the gas mixture was forced through the control chamber each day. The gases were obtained from cylinders of the Linde Air Products Company, and the required volumes were measured by the displacement of water in a graduated carboy. The prepared atmosphere was then forced through the control chamber by tap-water pressure. By this method the gas mixtures could be prepared with sufficient accuracy for these tests.

*Polarity and Variety Experiments.*—For experiments on the influence of polarity and variety on callus formation, and in other experiments in which optimum environmental conditions were desirable, moderately moist peat



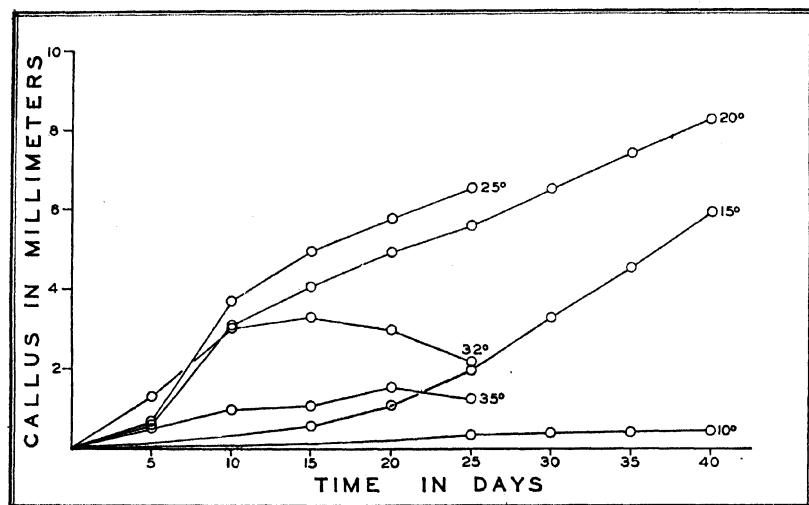
TEXT FIG. 1. Graphs showing the effect of temperature (degrees Centigrade) on the callusing of apple scion cuttings. The points of the curves, representing the average diameters of the callus rolls for individual cuttings, are based on four lots of material taken at five-day intervals. No callus formed at 0°, 5°, and 40° C. during the 40-day period.

moss was generally used as a medium. Flats, crocks, or other types of containers were used. The principal requisites for these tests were suitable temperature, moisture, and aëration to permit normal callus formation.

*Measuring Callus Development.*—In recording the amount of callus formed in the different experiments, readings were generally made on the basis of the diameter of the callus roll in millimeters. Since distinct differences exist in the amount of callus formed at the lip as compared with that formed on the side and base of slanting cuts, separate readings were made for these different areas. It was not considered possible or even essential to make these determinations precise. Two arbitrary values were



used for callus less than one millimeter; namely, a trace, recorded as 0.25 millimeter, and slightly less than one millimeter, recorded as 0.75 millimeter. No fractional values were recorded above one millimeter; whole values were used, as one millimeter, two millimeters, etc. In reading the callusing of a graft union, the graft was pulled apart and the amount of callus on the scion cut and on the stock cut was recorded as for the cuttings. In very advanced stages of callusing, it was sometimes difficult to determine the exact origin (scion or stock) of the callus, for it could not be expected that



TEXT FIG. 2. Graphs showing the effect of temperature (degrees Centigrade) on the callusing of apple root cuttings. The points of the curves, representing the average diameters of the callus rolls for individual cuttings, are based on four lots of material taken at five-day intervals. No callus formed at 0°, 5°, and 40° C. during the 40-day period.

by roughly breaking apart two connecting meristems all the scion callus would adhere to the scion piece and all the stock callus would adhere to the root piece. However, by combining the callus found on the two pieces, the total amount present at the union could be determined with sufficient accuracy.

## EXPERIMENTS AND RESULTS

### Effect of Temperature on Callus Formation

#### *Experiment 1 (Constant Temperatures)*

*Methods.*—Yellow Transparent scion cuttings and northern hardy seedling root cuttings were callused at the following constant temperatures: 0°, 5°, 10°, 15°, 20°, 25°, 32°, 35°, and 40° C. The purpose of the experiment was to study the specific effect of temperature on callusing. To observe these processes in detail it was necessary to use comparatively small numbers of cuttings; to offset the error that would necessarily result

TABLE 1. *Effect of Constant Temperatures on the Rate of Callusing of Scion and Stock Cuttings at Optimum Moisture (Callus per Individual Cutting as Total of Diameters of Roll Formed on Lip, Side, and Base of Slanting Cut)*

Time in Days	0° C.		5° C.		10° C.		15° C.		20° C.		25° C.		32° C.		35° C.		40° C.	
	Scion	Stock	Scion	Stock	Scion	Stock	Scion	Stock	Scion	Stock	Scion	Stock	Scion	Stock	Scion	Stock	Scion	Stock
3																		
4									(c) .25	.75	(d) .75	0	(a) 2.5	.75	.75	.75		
5									(b) .75	.75	.75	.75	.75	.75	.75	.5		
									(d) .75	.50	2.25	.75	2.25	1.75	2.25	.75		
6									(c) 2.25	.75	.75	.75	2.0	1.50	0	0		
7							(a) .25	0	(b) 3.0	.75	3.5	.75	4.0	4.0	1.5	1.25		
8									(d) 2.0	1.25	2.25	2.25	4.75	1.75	1.5	.75		
9									(c) 3.0	4.75	2.25	4.0	4.0	2.0	.75	.5		
10							(a) .75	0	(b) 3.0	.75	3.75	.75	7.0	6.0	2.0	1.75		
							(b) .25	0	(d) 6.0	3.0	4.0	5.0	4.25	2.25	2.0	1.5		
11									(a) 1.25	.5	5.0	5.0	7.0	6.0	2.0	2.0		
12							(c) .25	0	(b) 4.0	4.75	5.0	5.0	4.0	4.0	0	0		
14							(a) 1.5	.75	(d) 6.0	3.0	5.0	5.0	4.25	2.25	3.25	1.5		
15							(d) 0	.25	(c) 5.0	5.25	6.0	6.75	8.0	6.75	2.0	2.0		
16							(c) .25	.75	(b) 5.0	1.75	7.0	2.5	2.75	.5	1.5	.5		
17							(b) 1.5	0	(d) 4.0	4.25	5.0	4.75	4.0	4.0	0	0		
19							(a) .5	0	(c) 7.0	6.0	7.0	6.0	6.25	2.25	3.0	1.5		
23									(b) 6.0	5.75	9.0	7.0	10.0	6.0	2.25	4.0		
24							(d) 0	.25	(c) 9.0	2.5	9.0	6.0	4.0	.5	1.5	1.5		
25							(b) .75	0	(d) 7.0	4.75	7.0	6.0	4.0	0	0	0		
27							(a) 1.25	.75	(c) 8.0	9.0	8.0	6.0	7.0	1.5	4.0	1.5		
									(b) 10.0	5.25	11.0	7.0	4.0	1.75	2.0	1.0		
39							(d) .75	.5	(c) 12.0	9.0								
40							(c) 2.25	.25	(d) 10.0	9.0								
41							(b) 2.25	.25	(c) 10.0	9.0								
43							(a) 2.25	.75	(d) 10.0	6.25								

(a), Lot of 12/31/27. (b), Lot of 1/1/28. (c), Lot of 1/2/28. (d), Lot of 1/3/28.

from using a single cutting for each temperature, a similar series was started on each of four successive days. The containers were 21-centimeter ( $8\frac{1}{4}$ -inch) test tubes, and into each tube filled with moist peat moss one cutting was placed so that it was completely covered with the medium. The tube was then closed with a cork stopper having a very small aperture to permit some exchange of air. One tube for each scion and root cutting was placed at each of the nine temperatures, thus making a series of 18 tubes in all. One series was started on December 31, the second series on January 1, the third on January 2, and the fourth on January 3.

Examinations were made after 4, 7, 9, 11, 14, 19, 27, and 43 days. Readings were made very rapidly so as to reduce to a minimum the injury that might have resulted through exposure to the air. At each examination the general condition of the plant material was noted, as well as the amount of callus formed on the lip, side, and top of the slanting cut.

*Results.*—Table 1 shows the amount of callus formed per cutting, each value in the table having been obtained by totalling the diameters of the callus rolls on the lip, side, and base of the slanting cut. Averages for the four different lots at five-day intervals are plotted in text figures 1 and 2. It may be seen from table 1 that at 5° C. or below no callus formed during the 43-day period of the experiment. At 10° measurable callus had formed on the scion after 19 days and on the stock after 23 days, the amount increasing during the following three weeks at a fairly slow, uniform rate. At 15° callusing began after seven days on the scion and after 11 days on the stock. As shown in text figures 1 and 2, at 15° there was a gradual growth acceleration during the period, and by the 40th day the growth curve was still upward. At 20°, 25°, and 32° measurable amounts of callus had formed during the first five days. At these three temperatures growth was very rapid during the early part of the growth period; the rate at 25° was somewhat higher than at 20°, and the rate at 32° was slightly higher than at 25°. During the 40-day period the amount of callus reached a maximum and then decreased at both 25° and 32°, whereas the amount of callus continued to increase at 10°, 15°, and 20°. The time before the maximum volume was attained was shorter with a higher temperature. Callusing reached its greatest volume in approximately 20 days at 32° and 25 days at 25°. At 35° callus began to form promptly; it continued to increase slowly in volume for the first three weeks, but soon became browned on the surface and showed signs of injury. No callus was ever observed to form at 40° C.

*Discussion.*—The effect of temperature on the rate of callus formation is very striking. Temperatures falling between 0° and 40° C. represent the range of possible temperatures at which callus may form on detached shoot and root cuttings of the apple. It is very doubtful if callus ever would form at 0° with this type of material. At 5° no appreciable amount of callus formed during a period of approximately two months, but over a

period of from six months to one year distinct callusing did occur at this temperature (not shown in these data). At 10° callusing began very slowly, and after starting, usually showed little or no acceleration; there was merely a steady increase in volume of callus. For storage periods between one and two months 15° was found to be a good callusing temperature. At this temperature callus formation began more slowly than at higher temperatures but more rapidly than at 10°, and after an initial period of slow growth, a distinct acceleration occurred. (The characteristic appearance of grafts stored for six or seven weeks at 5°, 10°, and 15° C. is shown in Plate XXII, figure 2.) Rapid callusing occurred at 20°, 25°, and 32°, the rate being more rapid the higher the temperature. A maximum volume was attained at 25° after about four weeks, and at 32° after from two to three weeks; the volume then decreased at each of these temperatures. While the data suggest that a greater final volume of callus may be had at 20° or lower than at higher temperatures, this may not always be the case. A preferable interpretation of repeated trials would be that for callusing periods longer than two weeks, temperatures of 20° or lower will in general give callus that is healthier in appearance than that which develops at higher temperatures, and this improved condition of the callus may often result in greater abundance. At 35° callusing was never found to be satisfactory; the surface cells of the callus became brown and formed a layer of cork almost immediately, so that further growth of necessity must have come from within. At 40° death of the tissues occurred, being consistently followed by an abundance of mold.

For temperatures between 5° and 32° C., and for the initial part of the growth period, the rate of callus formation is greatly accelerated by a rise in temperature. In this respect the growth of callus resembles many chemical processes in which a rise in temperature of 10° C. doubles or trebles the rate of reaction. But for other temperatures, especially those above 32° C., and for the later phases of the growth period, no simple relation exists between temperature and rate of callus formation. Only within certain limits of time and temperature is the growth of callus accelerated with temperature rise and retarded with temperature fall.

In controlling the rate of callus formation by means of temperature, the result may more readily be understood if a callus roll be regarded as a colony of meristematic cells which undergoes a growth cycle like that of a colony of bacterial cells or like that of the plant of which the callus is a part. There are perhaps several stages of callus growth, as Buchanan (1918) has shown for a bacterial culture: an "initial stationary phase," a "positive growth acceleration phase," a "logarithmic growth phase," a "phase of negative growth acceleration," a "maximum stationary phase," and several death phases. While different temperatures specifically influence the duration of these different stages of growth as well as the rate of growth during each phase, the important point to be noted here is that

the formation of new callus cells does not continue indefinitely. A certain maximum volume of callus is reached, following which the callus tissues either disintegrate or are transformed into permanent tissues. With high temperatures this phase is attained very quickly, and unless the temperature is immediately lowered, injury and subsequent decay follow. In the field the plant as a whole grows concurrently with wound healing processes, and instead of decaying, the callus becomes protected by an external corky layer, internal growth continues, and the meristematic cells differentiate into permanent tissues. The result is frequently a callus overgrowth.

### *Experiment 2 (Constant Temperatures)*

*Methods.*—Wealthy cuttings were placed for a nine-day period at constant temperatures similar to those used in the previous experiment. Two sets of nine 500-cc. wide-mouth bottles were used as containers. In one set moistened filter paper was placed on the bottom of the bottles to maintain a high humidity; in the other, the cuttings were completely surrounded with moist peat moss. To permit an exchange of air the bottles were stoppered with absorbent cotton. In the filter-paper series it was possible to measure the amounts of callus formed without disturbing the cuttings; hence an examination of these was made after four days and an examination of both lots after nine days.

*Results.*—As shown in table 2, no callus formed at 0°, 5°, 10°, and 40° C. during the nine-day period. At 15° and 20° no callus formed during the first four days, but by the ninth day slight callusing had taken place at 15°

TABLE 2. *Effect of Constant Temperatures on the Rate of Callusing of Scion Cuttings at Optimum Moisture (Average Diameter of Callus Roll per Individual Cutting)*

Temperature ° C.	Average Callus per Cutting in Millimeters After 4 Days *	After 9 Days †
0.....	0	0
5.....	0	0
10.....	0	0
15.....	0	1.25
20.....	0	3.25
25.....	1.00	4.50
32.....	1.00	5.00
35.....	2.00	1.75
40.....	0	0

\* Filter paper series.

† Averages of both series.

and moderate callusing at 20°. At 25°, 32°, and 35°, callusing had occurred by the fourth day, being most rapid in its formation at the highest of these temperatures. By the ninth day, however, callusing at 25° and 32° had greatly increased, whereas at 35° injury had resulted in an actual decrease in callus.

*Discussion.*—While the results of experiment 2 covering the nine-day

period are not identical with those of experiment 1, they are sufficiently in accord to show the same general effect of temperature on the rate of callus formation. The time required for initiation and first appearance of callus shortens and the rate of subsequent formation increases with increasing temperature within the limits favorable for tissue growth. Above 32° injury occurred even during this short time.

### *Experiment 3 (Variable Temperatures)*

*Methods.*—Scion cuttings, root cuttings, and grafts of three apple varieties were callused at variable temperatures. The temperatures used were as follows: (a) 6°–14° C. (av. 9°), slowly rising temperature (outdoor storage cellar); (b) 13°–18° C. (av. 16°), slowly rising temperature (basement storage room); (c) 4°–19° C. (av. 7°), sharply fluctuating temperature (cold frame); (d) 14°–23° C. (av. 18°), sharply fluctuating temperature (greenhouse).

The slowly rising temperatures were recorded by a thermograph and showed a gradual rise from the lowest reading to the highest over a period of 52 days. The sharply fluctuating temperatures were recorded by daily readings. In the cold frame at this time of the year (spring) the temperature rose sharply during the short mid-day period and then dropped rapidly again, remaining comparatively low for the balance of the 24-hour period. The average temperature, based on readings taken at approximately nine o'clock each morning, was only 7° C. The greenhouse temperatures were subject to somewhat the same solar influence. Steam heat prevented the temperatures from ever dropping below a fixed point, but about mid-day a rather sharp rise and fall of temperature occurred due to the sun.

Moisture in this experiment may be regarded as fairly constant since all the plant material was placed in peat moss of nearly the same water content. (It is shown in a later section of this paper that the moisture content of peat may vary greatly without an observable effect on the callusing.)

One flat containing the following plant materials in peat moss was subjected to each of the four variable temperatures:

		Number of Pieces
Jonathan:	Scion cuttings.....	10
	Grafts (variously wrapped).....	30
Wealthy:	Scion cuttings.....	10
	Grafts (variously wrapped).....	30
Yellow Trans- parent:	Scion cuttings .....	10
	Grafts (variously wrapped).....	30
French crab root cuttings.....		10

By the term "variously wrapped" is meant that some of the grafts were wrapped with waxed thread, some with clean muslin cloth, and some

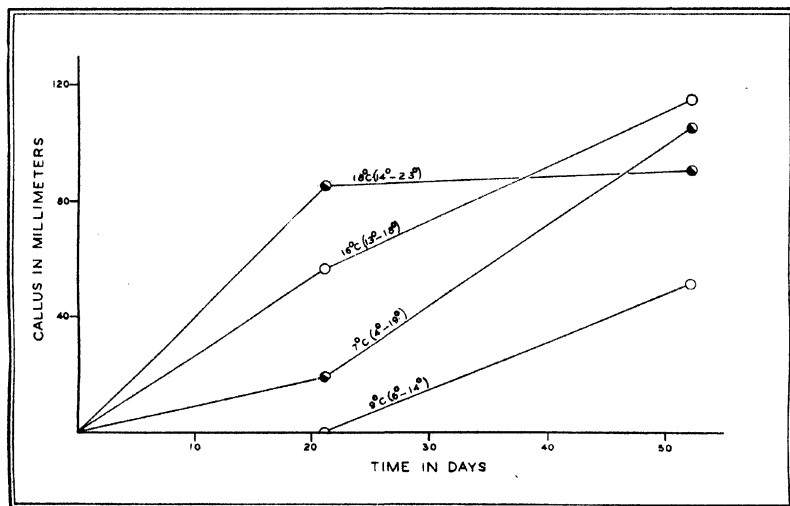
with friction tape. The data for these methods of wrapping are grouped, since the question of wrappers is not to be discussed in this paper.

The material was examined after 21 days and after 52 days. At each of these times from two to five pieces were taken at random from each bundle of plant material. The rest of the bundle was left undisturbed, and the data were recorded rapidly, so as to interrupt growth processes as little as possible.

*Results.*—Table 3 and text figure 3 show that with the slowly rising temperature beginning at 6°, no callus had formed after three weeks, and that with the corresponding slowly-rising temperature beginning at 13° C. only a moderate amount of callus had formed at this time. However, at

TABLE 3. *Effect of Variable Temperatures on Rate of Callusing of Apple Cuttings and Grafts*

Time in Days	Total Callus in Millimeters			
	Slowly Increasing Temperature		Sharply Fluctuating Temperature	
	0° C. (6°–14°)	16° C. (13°–18°)	7° C. (4°–19°)	18° C. (14°–23°)
21	0	57.0	19.0	86.0
52	54.0	115.0	104.0	91.0



TEXT FIG. 3. Graphs showing the effect of variable temperatures (degrees Centigrade) on the callusing of apple cuttings and grafts. The points of the curves are based on the total amount of callus formed, obtained by combining the diameters of the callus rolls for all plant individuals used, the number of pieces for the four different lots being comparable. Both the average temperature for the storage period and the range of variation (in parentheses) are shown.

the end of 52 days, when these temperatures had risen to 14° and 18° C., respectively, moderate callusing had occurred at the lower temperature (about equalling the amount formed at the higher temperature in 21 days), and abundant callusing had occurred at the higher temperature.

With the sharply fluctuating temperature ranging from 4° to 19°, the average temperature was low (7°), but the sudden daily rise in temperature was sufficient to cause a slight callusing after 21 days and abundant callusing after 52 days. In the higher range of sharply fluctuating temperatures (14°–23°) callusing took place under greenhouse conditions, and nearly reached its greatest volume in three weeks.

*Discussion.*—This experiment again illustrates the point that the rate of callus formation increases with rise in temperature. Within certain limits any rise in temperature appears to accelerate the processes of cell division and cell enlargement and any fall in temperature results in retardation of these processes. Even though a fairly uniform temperature be maintained, any variation from this temperature, even for short periods of time, has a noticeable effect on the volume of callus obtained after a given period of time. The fact that in the range of 14°–23° the amount of callus at the end of 52 days was less than that in the ranges of 13°–18° and 4°–19° was apparently due to unknown conditions in this experiment, and should be regarded as exceptional rather than normal.

#### *Temperature as an Aid in Overgrowth Control*

These data not only substantiate the broad generalization that low temperatures retard growth processes and high temperatures accelerate them, but show that callus formation may be regulated as desired by the proper manipulation of temperature. Any tendency toward overgrowth formation during the storage period may be immediately checked by reducing the temperature to about 3° C. This retardation may be only temporary, however, for when the grafts are transferred to field conditions, growth processes may continue. Nevertheless, tongue grafts which have been well callused before planting have only rarely shown the beginnings of an overgrowth at the union after one season in the field. While these grafts were prepared with some care, no attempt was made to select scion and root pieces of equal size or to match precisely the cut surfaces. It may be that a proper regulation of the callusing of root-grafts prior to planting by means of temperature control will prove important in the prevention of overgrowths.

### **Effect of Temperature and Moisture on Callus Formation**

#### *Experiment 4 (Temperature and Moisture)*

*Methods.*—Scion cuttings, root cuttings, and grafts of three apple varieties were callused under conditions of varying temperature and moisture. Averages of the temperatures used were 4°, 9°, and 16° C.



None of these temperatures was strictly constant. The 4° temperature was maintained by refrigeration and occasionally dropped to 0°. The 9° temperature (temperature *a*, experiment 3) was obtained in an outdoor storage cellar, the thermograph record showing a very gradual rise from 6° to 14°. A basement storage room was used for the 16° temperature experiment (temperature *b*, experiment 3), the thermograph record showing a gradual rise from 13° to 18° from March until early June.

Moisture was controlled by means of sulfuric acid solutions, and for each of the three temperatures a series of five humidities was provided, these being expressed as 20, 40, 60, 80, and 100 percent relative humidity. However, in an experiment of this kind the important factor to be controlled is the saturation deficit; that is, the difference between the vapor tension of water at a given temperature and the aqueous tension of a solution of sulfuric acid at that temperature.

Since the vapor pressure curve of water and that of the sulfuric acid solutions diverge from each other as the temperature increases, a solution which will establish a certain saturation deficit at one temperature will no longer do so at another temperature, and in order that the same saturation deficit may be established at both temperatures, solutions of different concentration must be used. The following table shows the saturation deficits corresponding to each of the relative humidities at the three temperatures.

Percent Relative Humidity	Saturation Deficit (mm. of Hg) <sup>1</sup>		
	4° C.	9° C.	16° C.
20	4.88 <sup>a</sup>	6.89	10.90
40	3.66 <sup>b</sup>	5.17 <sup>a</sup>	8.18
60	2.44	3.44 <sup>b</sup>	5.45 <sup>a</sup>
80	1.22	1.72	2.73 <sup>b</sup>

<sup>1</sup> Vapor pressure data from International Critical Tables, volume III. Nearly comparable saturation deficits are provided at different relative humidities, designated by letters *a* and *b*.

Although the experiments were not planned so as to obtain the same series of saturation deficits (from low to high) with each of the three temperatures, nevertheless, for each temperature a considerable range of saturation deficits was provided.

The general procedure was as follows: A large glass crystallizing dish (25 cm. × 12.5 cm.) was placed on the bottom of a 12-gallon glazed crock. Approximately two liters of sulfuric acid solution were placed in the crystallizing dish. The concentrations were carefully prepared with reagent quality acid, according to Wilson's (1921) vapor pressure chart. A circular screen made of heavy galvanized wire was placed on the crystallizing dish, and over this a double thickness of cheese-cloth to prevent soil or plant

particles from falling into the acid. The plant material was then placed in the container. A flanged lid covered the crock and this was sealed airtight with plasteline (modeling wax). Altogether, 15 similar crocks were used to provide the five humidities at three temperatures. An additional crock, in which the plant material was placed over water, was similarly prepared, this crock remaining unopened for 45 days to show if opening the containers to examine plant materials seriously affected the results.

The plant material placed within each crock was as follows:

		Number o. Pieces
Jonathan:	Scion cuttings.....	25
	Grafts (variously wrapped) <sup>2</sup> .....	60
Wealthy:	Scion cuttings.....	25
	Grafts (variously wrapped).....	60
Yellow Trans- parent:	Scion cuttings.....	25
	Grafts (variously wrapped).....	60
French crab root cuttings.....		25

<sup>2</sup> See experiment 3.

Examination of the plant material was made at the end of 14, 28, and 49 days. At each of these times the crocks were opened and from two to five pieces taken at random from each bundle. Callusing was examined, observations recorded, and the pieces discarded.

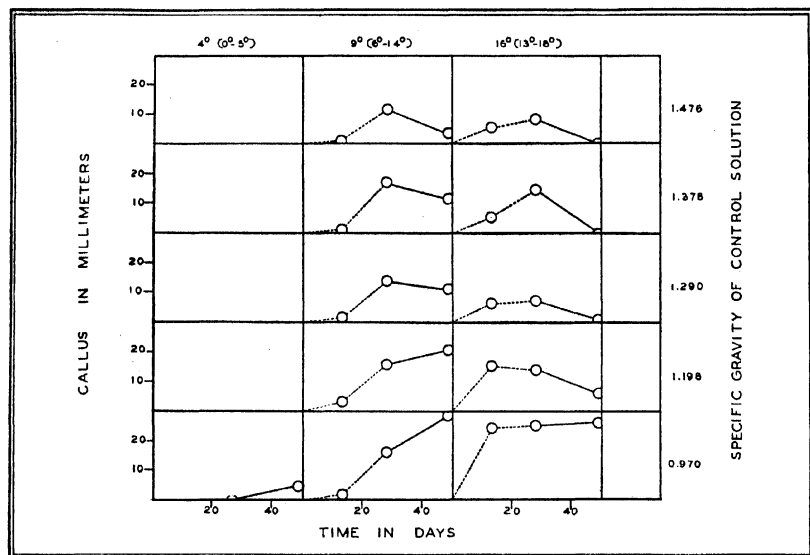
*Results.*—The amount of callus formed at humidities below saturation was always small, and even at saturation callusing was much less abundant than that obtained in other tests in which the plant materials were in contact with moist peat moss. Table 4 and text figure 4 show that over water at

TABLE 4. *Effect of Temperature and Moisture on the Callusing of Apple Cuttings and Grafts*

Initial Specific Gravity of Acid	Initial Percent Relative Humidity	Total Callus in Millimeters								
		4° C. (0°-4°)			9° C. (6°-14°)			16° C. (13°-18°)		
		14 Days	28 Days	49 Days	14 Days	28 Days	49 Days	14 Days	28 Days	49 Days
1.476	20	0	0	0	1.75	10.75	3.75	5.50	7.25	0
1.378	40	0	0	0	0.75	16.50	11.25	5.00	14.25	0
1.290	60	0	0	0	2.00	12.75	10.50	5.25	7.00	1.50
1.198	80	0	0	0	2.75	15.25	20.25	15.25	14.75	6.75
0.970	100	0	0	4.50	2.50	17.00	27.75	24.25	25.25	25.75

4° C. only a trace of callus had been formed at the end of 49 days, and that no callus whatever had been formed at the other humidities. At 9° callusing began very slowly, but after two weeks a sharp acceleration took place due to the steadily rising temperature. In this temperature range slight callusing was had over all acid solutions; but over the three strongest

solutions the callus which had formed during the early period of the experiment was later partially destroyed through desiccation, the rate and extent of desiccation increasing with the acid concentration. Callus formation at the end of 14 days at 16° C. was more abundant in every case than at the



TEXT FIG. 4. Graphs showing the desiccation effect of combined temperatures and humidities on callusing cuttings and grafts. Though slight callusing (totals shown) occurred over acid solutions, particular attention is called to the curves showing decrease in callus due to desiccation (represented by a solid line). The average temperatures (degrees Centigrade) for the storage period and the ranges of variation (in parentheses) are shown. Since relative humidities were not constant in all cases, they are represented by the initial specific gravities of the solutions.

lower temperature ranges, this difference being most pronounced over water and the dilute acid. As in the test at 9°, early-formed callus seems to have disintegrated more quickly with increased concentration of acid. There was little evidence of injury to the callus through opening the containers for examination.

*Discussion.*—In general, callus formation under the inclosed conditions of this experiment was at the most no more than fair, being neither uniform nor especially abundant in any particular case.

The fact that some callusing did take place over acid solutions of sufficient initial strength to provide very low humidities should not be construed to mean that callus really is able to form if the plant materials are surrounded by air in which these humidities are maintained. It would seem that the explanation lies in failure of the acid to establish an equilibrium quickly. This seems probable from a consideration of the experimental

conditions under which these tests were made, and it is supported by the results of experiment 5, described later. The moisture present in the air of the container at the start was so small in amount that it may be disregarded; a sufficient quantity of it could have been taken up by the acid solution quickly enough to establish an early equilibrium. The plant material placed within the containers, however, was of sufficient quantity to provide a large supply of available water. It seems probable that within a short time considerable water had been evaporated from the plant material and absorbed by the acid solution, and that this water served to dilute the acid solution materially and tended especially to form surface layers of much lower concentration than the underlying mass of solution. In one experiment in which the cuttings were placed over different concentrations of sulfuric acid it was found that at the end of six days they had lost weight as follows:

Initial Specific Gravity of Acid	Percentage Loss in Weight
1.476.....	17.2
1.378.....	12.2
1.290.....	9.6
1.198.....	5.7
0.970.....	0.9

At this rate, estimating the weight of plant material in each container as 2400 grams, 412 cc. of water conceivably may have been withdrawn from the plant material over the most concentrated acid during the first six days. This amount of water would be sufficient to form a watery layer which could greatly modify the drying power of the acid solution. The formation of such a layer would be possible because neither the air in the containers nor the acid solutions was in motion, and considerable time would be required to establish equilibrium through diffusion and convection currents. During this initial period it would be possible that those cuttings most protected by a surrounding mass of plant material could actually callus slightly. If a smaller volume of plant material had been used, and if this material had been separated instead of being placed in bundles in which centrally-located cuttings were more or less protected, it is quite unlikely that callus would have formed over the acid solutions with a specific gravity of 1.476, 1.378, and 1.290.

The effect of these humidities at the different temperatures can be seen more clearly (text fig. 4) where the callus curves show the rate and extent of disintegration rather than the rate of formation and final volume attained. It is likely that equilibrium had been established by that time. During this late phase of the storage period it may be seen that all the slopes of the curves are downward, indicating desiccation, with the exception of the lots in saturated atmospheres and the lot over the most dilute acid at 9° C. All solutions of acid, except in one case, brought about desiccation of the callus of cuttings and grafts, and the rate of desiccation increased

with the acid concentration. Further, desiccation was more marked throughout at the higher temperature.

#### *Experiment 5 (Relative Humidity)*

*Methods.*—Since with sulfuric acid solutions of the preceding experiment the concentrations became modified by the absorption of moisture from the plant material, it seemed desirable to employ other means of maintaining humidity in which this source of error would be eliminated. Hence, an experiment was begun in which apple cuttings were callused in a continuous current of air, the humidity of which was controlled by means of saturated solutions of inorganic salts.

A number of salts were selected to provide a wide range of humidities. All solutions were made of "C.P." salts and distilled water. In the process of preparation, each solution was agitated by a motor-driven stirrer for a period ranging up to four hours in length, while the salt was added as rapidly as it was dissolved. At the same time the temperature of the solution was maintained slightly above that of the room so that on cooling saturation would be assured (one of the salts, calcium sulfate, is slightly more soluble in the cold). The solutions used with the relative humidities delivered, as determined by means of a special humidity testing instrument (Shippy, 1929), were as follows:

Solution	Percentage Relative Humidity
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ .....	56
$\text{NaNO}_3$ .....	66
$\text{NH}_4\text{Cl}$ .....	79
$(\text{NH}_4)_2\text{SO}_4$ .....	81
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ .....	90
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ .....	95
$\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ .....	96
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ .....	98
Water.....	100

The influence of temperature on vapor pressure and saturation deficit may be disregarded in this experiment, since all relative humidities were provided at the same temperature.

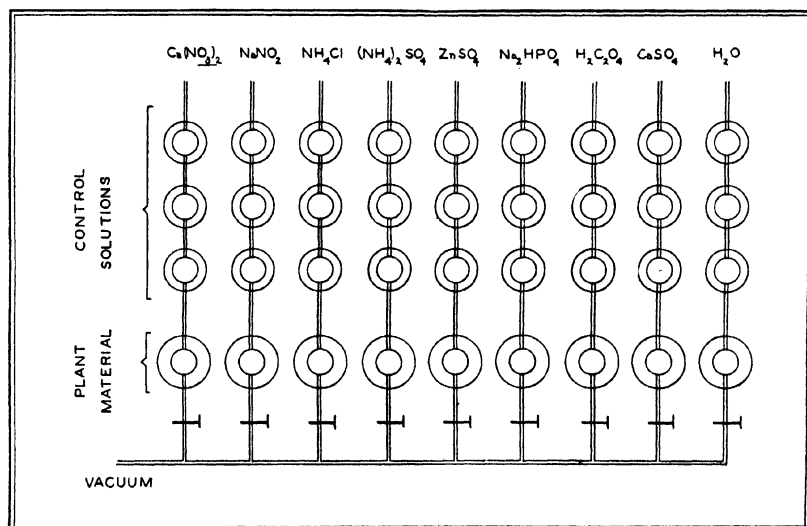
Text figure 5 shows the arrangement of the apparatus. Ten series of four bottles each (one series for each solution) were so arranged that they could be connected to a single vacuum outlet. Three of the bottles were of 500 cc. capacity and each contained about 375 cc. of the solution. The fourth bottle held the cuttings and was of one liter capacity. The vacuum pull was regulated by means of clamps so that a slow, fairly constant flow of air passed through the solutions and containers holding the cuttings.

Plant material for the experiment consisted of ten Wealthy apple cuttings for each humidity, making a total of 90 cuttings. Callusing was recorded after six days at room temperature (20°–25° C.).

**Results.**—As shown in table 5, during the six-day period the most callus

TABLE 5. *Effect of Relative Humidity on Callusing of Wealthy Cuttings*  
(Temperature 22°–25° C.)

Solution	Percent Relative Humidity	Comparative Amount of Callus
Calcium nitrate.....	56	0
Sodium nitrite.....	66	+
Ammonium chlorid.....	79	+
Ammonium sulfate.....	81	++
Zinc sulfate.....	90	++
Disodium phosphate.....	95	+++
Oxalic acid.....	96	+++
Calcium sulfate.....	98	+++
Water.....	100	+++
+ Trace.		
++ More.		
+++ Most.		



TEXT FIG. 5. Diagram showing the arrangement of equipment for providing a graded series of relative humidities by the use of saturated solutions of inorganic salts. Each vessel (bottle) is represented by two concentric circles, and the tubing used to connect the different series to the vacuum is indicated by solid lines. The small T's show where clamps were attached.

formed between 95 and 100 percent relative humidity, less between 80 and 90 percent, still less between 66 and 79 percent, and no callus formed at 56 percent.

**Discussion.**—In this experiment, as in the preceding, callus formation was only fair even at humidities close to saturation. Apparently even in

the case of water-saturated air, the moisture condition was not optimum, since at the close of the experiment there appeared no likelihood that the cuttings would callus further.

#### *Experiment 6 (Moisture Tolerant)*

*Methods.*—Since moistures below 100 percent relative humidity had failed to give uniform, abundant callusing, in this experiment 100 percent relative humidity was provided in every case and the moisture variation was that of the total water content of the callusing medium (peat moss). Hence, this experiment represented a test under variable temperatures of the tolerance of callus to environments in which liquid water was increasingly available.

Temperatures of 10°, 15°, 20°, and 22°–25° C. were used. The water content of the peat moss medium in which the cuttings were placed varied as follows: 97, 177, 271, 337, and 437 percent (averages for period on basis of 100 parts oven-dried peat moss). The plant material was as follows:

#### Scion cuttings:

Yellow Transparent  
Willow Twig  
Wolf River  
Ben Davis  
Delicious

#### Root cuttings:

Austrian crab

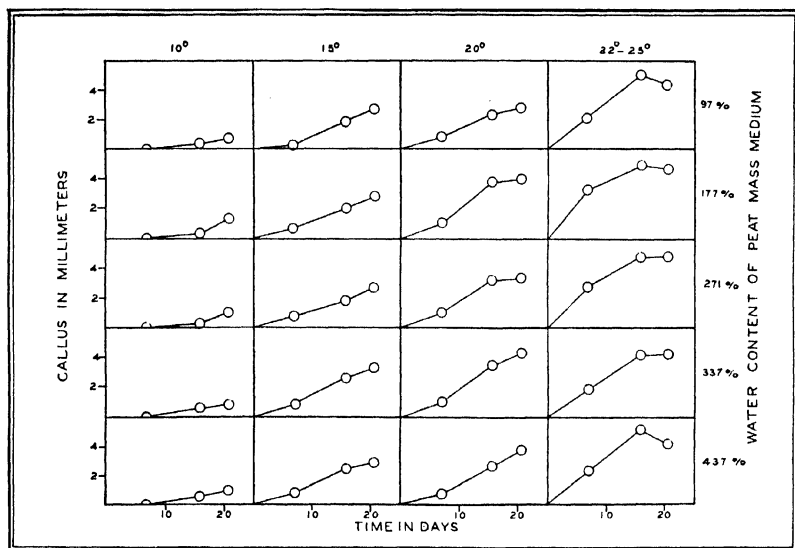
The four temperatures and the five moistures made a total of 20 different sets of conditions. Fifteen cuttings of every variety were used for each condition. All cuttings were first cut with hand shears, and then the ends were trimmed with a sharp knife to ensure a smooth surface. Containers consisted of deep flats (depth, 4 inches) divided into three compartments by lengths of lath. In preparing a flat, a one-inch layer of peat moss was spread over the bottom; the cuttings were placed upon this, and then were covered with enough peat moss to fill the flat. Fifteen flats were used for each temperature, or three for each of the five different moistures.

Examinations were made after 7, 16, and 21 days. Usually three cuttings were picked at random for callus readings, the others remaining undisturbed.

*Results.*—Table 6 and text figure 6 show that the moisture content of the peat moss medium may vary between wide limits without influencing the rate of callus formation. Considerable moisture tolerance is indicated by the fact that callus formed almost equally well in media varying in water content from 97 to 437 percent (Pl. XXIII, figs. 1–5). Further, these results indicate that once the moisture content of the medium is such that the air within the medium is saturated, additional quantities of water have little or no stimulating effect on callus formation.

TABLE 6. *Effect of Temperature and Moisture on Callusing of Apple Cuttings*

Water Content of Peat Moss Medium, Percent	Average Callus per Cutting in Millimeters											
	10° C.			15° C.			20° C.			22°-25° C.		
	7 Days	16 Days	21 Days	7 Days	16 Days	21 Days	7 Days	16 Days	21 Days	7 Days	16 Days	21 Days
97	0	.4	.70	.25	1.9	2.8	.8	2.3	2.9	2.2	4.8	4.4
177	0	.4	1.25	.70	2.0	2.9	1.0	3.8	4.0	3.2	4.8	4.6
271	0	.4	1.00	.70	1.7	2.6	1.0	3.2	3.3	2.7	4.7	4.7
337	0	.6	.90	.80	2.5	3.3	1.0	3.4	4.4	1.8	4.2	4.3
437	0	.5	1.00	.80	2.6	3.0	.7	2.6	3.8	2.4	5.2	4.4



TEXT FIG. 6. Graphs showing the combined effect of temperature and liquid moisture on the callusing of apple cuttings. The amount of callus is expressed as the average diameter of the callus rolls per individual cutting. Temperature is shown in degrees Centigrade, and moisture is expressed as the total water content of the peat moss medium (percentage water by weight per 100 parts oven-dried peat moss).

*Discussion.*—The conditions afforded in this experiment, as well as others in which cuttings and grafts have been callused in moist peat moss, permitted uniform callusing that had never been obtained when the material was merely exposed to a moist atmosphere even though the air were almost saturated with water vapor. This has seemed to indicate the desirability of actually having liquid moisture in contact with the cuttings, as is the case in a moss or sand medium. The liquid moisture perhaps need be no more than a film, for good callusing has been had repeatedly in a medium



only slightly moist to the touch; in fact, in a medium in which the cuttings have actually decreased in weight due to water loss. However, if the medium is so dry that the loss of water from the cuttings is great, no callus forms. An experiment carried out with cuttings of Jonathan, Wealthy, and Yellow Transparent gives some idea of the water gain or loss from cuttings placed in a peat moss medium (table 7). The moisture contents

TABLE 7. *Water Gain or Loss of Cuttings and Peat Moss Callusing Medium*

	Initial Percent Water of Peat	Percent Gain or Loss of Water After 33 Days	
		Medium	Cuttings
A. No callus.....	16	+22	-26
	16	+19	-23
	17	+21	-31
	17	+16	-24
	17	+13	-25
	19	+13	-21
	19	+15	-23
B. Good callusing.....	98	- 3	- 3
	109	-16	- 4
	113	-14	- 6
C. Good callusing.....	230	-16	+ 3
	238	-31	+ 4
	252	-43	+ 3
D. Good callusing.....	405	-32	+ 5
	408	-46	+ 3
	418	-52	+ 3

- Loss in weight.

+ Gain in weight.

of groups *B*, *C*, and *D* are all sufficiently high to permit callusing, but the low moisture contents of group *A* so desiccated the cuttings that callusing could not take place. It seems that for normal callusing the cutting material must be held reasonably close to its original moisture content.

### Effect of Aëration on Callus Formation

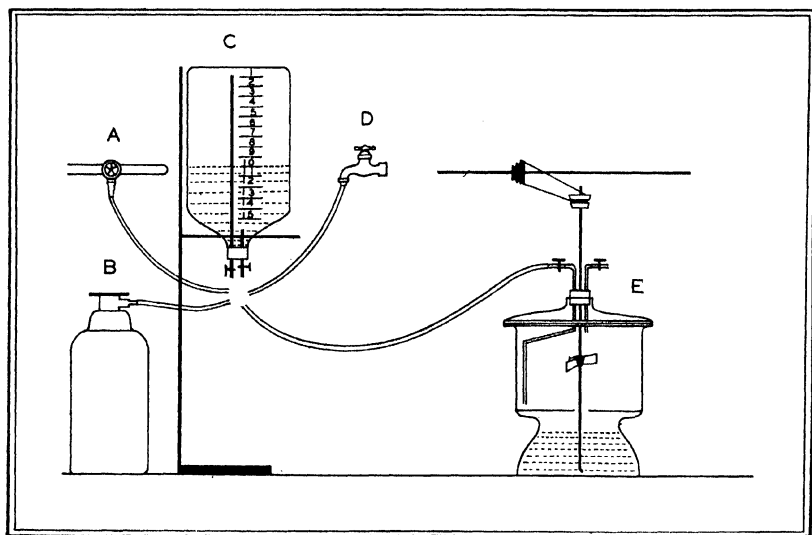
#### *Experiment 7 (Aëration)*

*Methods.*—Cuttings were callused under conditions of varying proportions of oxygen and air, as follows:

#### *Approximate Proportions in Percentage*

Oxygen	Air		Oxygen	Nitrogen
0	100	=	20	80
33	66	=	46	54
66	33	=	73	27
100	0	=	100	0

Text figure 7 illustrates the apparatus. The cuttings were placed in four large desiccators provided with tubulated covers, closed with three-hole rubber stoppers. Through the central hole a solid glass rod was passed having a fan attachment, which when rotated by means of power supplied by an electric motor, constantly kept the air in motion in the desiccator. Through the other two holes 6 mm. ( $\frac{1}{4}$  inch) glass tubing was passed by means of which the atmosphere in the desiccator was changed



TEXT FIG. 7. Diagram showing the arrangement of an apparatus for measuring and transferring to a desiccator various mixtures of oxygen and nitrogen or oxygen and air. The equipment includes (A) compressed air tap, (B) oxygen or nitrogen cylinder, (C) inverted, graduated carboy, (D) water faucet, and (E) desiccator used as control chamber. The proper volume of each gas is admitted to the water-filled carboy through the long tube of the carboy, the displaced water escaping through the short tube. By connecting the short tube with the water faucet and the long tube with that connected with the desiccator, the gas may be forced into the desiccator by tap-water pressure. As shown, the gas enters the lower part of the desiccator, displacing that previously present, which escapes through the short tube at the top.

without disturbing the other contents. Proportions of oxygen and air were measured in a calibrated, inverted carboy by displacing tap water by oxygen from a cylinder of the gas or by air from a compressed air outlet. The gas mixture was then forced into the desiccators by water pressure from the tap. To maintain the original gas mixture ten liters were forced through the desiccators daily in the manner described.

Plant material consisted of apple cuttings of unknown variety cut from trees growing on the premises of the Boyce Thompson Institute. Thirty-four cuttings were placed in each desiccator. Examination was

made after the preparations had remained for five and eight days at room temperature (approximately 25° C.).

*Results.*—Table 8 shows that callus formed on cuttings placed in high concentrations of oxygen, but indicates that pure oxygen inhibited its formation.

TABLE 8. *Effect of Oxygen on the Callusing of Scion Cuttings*

Time in Days	Percentage Oxygen	Callus
8.....	20	++++
5.....	46	+++
5.....	73	++
5.....	100	+

(+) One cutting showing callus.

(++) Several with callus.

(+++ ) Many with callus.

(++++ ) Majority with callus.

*Discussion.*—Callusing was clearly inhibited by 100 percent oxygen. With the other concentrations of oxygen the callusing showed great variability. Although table 8 suggests an inverse relation between callusing and oxygen concentration between 20 and 73 percent, this relation was not confirmed when the tests were repeated, in experiment 8.

#### *Experiment 8 (Aëration)*

*Methods.*—Both apple and privet cuttings were callused under conditions similar in all respects to those of experiment 7. The experimental period was 15 days, and 14 cuttings were used for each gas mixture.

*Results.*—Table 9 shows that callus may form over a wide range of oxygen concentrations, but pure oxygen again appeared to be inhibiting in its effect. (See Plate XXIII, figure 6, for injury to cuttings exposed to high oxygen concentrations.)

TABLE 9. *Effect of Oxygen on the Callusing of Apple and Privet Cuttings*

Percentage Oxygen	Callus in Millimeters*	
	Apple	Privet
20.....	3.3	3.0
46.....	2.1	2.3
73.....	3.8	2.3
100.....	0.8	0.0

\* Average per individual cutting.

*Discussion.*—In this experiment little difference in volume of callus occurred in oxygen concentrations ranging from that of air to that of an atmosphere containing 73 percent. At 100 percent oxygen, as in the previous experiment, callusing was inhibited.

#### *Experiment 9 (Aëration)*

*Methods.*—Apple cuttings of unknown variety were callused in concentrations of oxygen lower than normal air, as follows:

*Approximate Proportions in Percentage*

Air	Nitrogen	=	Oxygen	Nitrogen
0	100	=	0	100
20	80	=	4	96
30	70	=	6	94
40	60	=	8	92
50	50	=	10	90
60	40	=	12	88
70	30	=	14	86
80	20	=	16	84
90	10	=	18	82
100	0	=	20	80

The cuttings were placed in calibrated 40 cm. tubes fitted with fine-mesh copper screen supports inside to hold the cuttings above the water level. Cuttings were placed in the tube, and then the copper screen support was forced up into the tube about 5 cm. The tube was filled with water and inverted with the open end held beneath the water. The water was displaced by the proper amounts of compressed air and nitrogen, the tube being so calibrated that a column of water from 2.5 to 5 cm. deep remained inside to supply a high humidity. The tube was closed with a rubber stopper, and then placed at 15° C. The final volume of callus was obtained after 16 days.

*Results.*—The results given in table 10 show that normal callusing occurs when the oxygen supply is 14 percent or higher. Concentrations of oxygen from 6 percent to 12 percent permitted callus formation and a slight amount of callus formed even in 4 percent oxygen. Any concentration of oxygen below 12 percent was inhibiting in its effects.

TABLE 10. *Effect of Decreased Amounts of Oxygen on the Callusing of Apple Cuttings*

Percentage Oxygen	Callus in Millimeters *
0.....	0.00
4.....	0.25
6.....	3.00
8.....	3.00
10.....	3.00
12.....	3.00
14.....	5.00
16.....	5.00
18.....	5.00
20.....	5.00

\* Average per individual cutting.

*Discussion.*—This experiment is subjected to the criticism that since the gas mixture was not renewed from time to time its composition was different at the close of the experimental period from what it was at the beginning.

While this is doubtless true, it is equally safe to say that the oxygen concentrations were never greater than at the beginning of the experiment. These data are in accord with similar experiments showing that callusing does not take place in the absence of oxygen, but that smaller amounts than present in air are sufficient. In the absence of oxygen it is probable that respiration and other metabolic processes are so retarded that cell division, essential for the formation of callus, soon comes to a standstill.

Carbon dioxide, chiefly formed as a product of respiration, must be taken into consideration. In one experiment, cuttings of different ages were placed in pure carbon dioxide as well as in a mixture of equal parts of carbon dioxide and air. In no case (after 18 days) did callus form, whereas moderate callusing took place with similar lots of cuttings in various proportions of oxygen and nitrogen. In another experiment cuttings were placed in a graded series of high carbon dioxide concentrations. After 15 days callus had formed in air and in a mixture containing 10 percent  $\text{CO}_2$  and 90 percent air, but no callus had developed in mixtures containing higher percentages of carbon dioxide. Of course, in mixtures of carbon dioxide and air it is possible that oxygen would be a limiting factor. However, it seems more probable that carbon dioxide, in high concentrations, would reduce respiration and thus inhibit callusing.

### Effect of Polarity on Callus and Overgrowth Formation

It was early recognized in these experiments that both poles of apple cuttings do not callus equally. It was very evident that the bottom or lower ends of both scion and root cuttings callus the better, and this fact would seem to have an important bearing on the usual formation of overgrowths from the bottom of the scion.

#### *Experiment 10 (Polarity)*

*Methods.*—See experiment 6. It may be noted, however, that the only requirement to illustrate polarity is an environment permitting normal callus formation.

*Results.*—Distinct polarity was observed in apple scion (Pl. XXIII, figs. 1-5) and root stock cuttings with respect to the amount of callus which formed at opposite ends of the same cutting. Tables 11 and 12 show that callus was formed in much greater abundance on the bottom ends than on the top ends of both scion and root cuttings. This difference occurs regardless of temperature. Not every cutting calluses more abundantly from the bottom end, for individual variations do occur (where some factor, such as injury or localized depletion of water, acts to change the usual result). Observations of hundreds of cuttings, however, leave no doubt as to a marked difference in callusing capacity between the two poles of the same cutting.

TABLE 11. *Abundance of Callus Formed on Top and Bottom Ends of Apple Scion Cuttings \**

Time in Days	Number of Readings	Storage Temperature	Total Callus in Millimeters	
			Top	Bottom
7	47	15°	0.25	33.25
	71	20	3.50	58.50
	63	22-25	25.75	126.50
16	35	10°	0.00	18.50
	72	15	13.75	147.25
	65	20	33.75	162.50
	61	22-25	76.00	212.00
21	64	10°	2.50	61.50
	70	15	30.75	172.00
	71	20	48.75	177.75
	70	22-25	85.25	182.75

\* The terms "top" and "bottom" apply with respect to the ground line.

TABLE 12. *Abundance of Callus Formed on Top and Bottom Ends of Apple Root Cuttings \**

Time in Days	Number of Readings	Storage Temperature	Total Callus in Millimeters	
			Top	Bottom
7	2	20°	0.00	0.50
	10	22-25	1.75	7.25
16	8	15°	0.50	4.25
	10	20	2.00	9.75
	10	22-25	9.75	21.50
21	6	10°	0.00	3.25
	13	15	3.75	18.75
	15	20	4.50	23.50
	10	22-25	8.25	26.25

\* The terms "top" and "bottom" apply with respect to the ground line.

### *Experiment 11 (Polarity)*

Polarity is distinct in the case of the slanting cut made on the basal end of scion cuttings prepared as in making the tongue graft.

*Methods.*—See experiment 3. A favorable callusing environment is the only requirement.

*Results.*—Tables 13 and 14, representing two different tests, show that the lip produces callus far more abundantly than the other areas of the

TABLE 13. *Callus Formation from the Lip, Side, and Top of Slanting Cut Made on Basal End of Scion Cuttings*

Time in Days	Number of Readings	Total Callus in Millimeters		
		Lip	Side	Top
21	48	69	39	41
52	57	137	63	76

TABLE 14. *Callus Formation from the Lip, Side, and Top of Slanting Cut Made on Basal End of Scion Cuttings*

Time in Days	Number of Readings	Total Callus in Millimeters		
		Lip	Side	Top
18	95	124	66	64
52	94	266	112	130

slanting cut, whereas no marked differences exist between the base and the side. That the dominance of lip callusing is a polarity phenomenon is shown by the fact that similar slanting cuts made on the top end of the root piece show no striking dominance of the lip. In this case the lip, side, and base develop practically the same amount of callus.

#### *Experiment 12 (Polarity)*

The question may be raised as to whether or not the position of the cuttings with reference to gravity during the callusing period influences the effects of polarity.

*Methods.*—Wealthy and Yellow Transparent grafts were callused in upright, horizontal, and inverted positions in a medium of moderately moist peat moss at approximately 20° C. Callusing was examined after 34 days.

*Results.*—Table 15 shows that callusing was the same, whether the

TABLE 15. *Effect of Position of Apple Grafts During Storage on the Abundance of Callus \**

Variety	Position of Grafts	Scion Callus			Root Callus		
		Lip	Side	Top	Lip	Side	Top
Wealthy	Upright	4.0	2.0	2.0	1.0	0.75	1.0
	Horizontal	4.0	2.0	2.0	0.75	0.00	0.75
	Inverted	4.0	2.0	2.0	1.0	0.75	1.0
Yellow Transparent	Upright	4.0	0.75	2.0	0.75	0.0	1.0
	Horizontal	3.0	2.0	2.0	1.0	0.0	1.0
	Inverted	4.0	0.75	2.0	1.0	0.0	1.0

\* Average per individual cutting.

grafts were upright, horizontal, or inverted. The grafts united well in most cases, without reference to position. If gravity were responsible for the basally dominant development of callus, it must have acted prior to the callusing period.

*Experiment 13 (Polarity)*

Next to the possibility of a gravitational causation for basal dominance, that of food accumulation might be considered. This has not been given particular study in these experiments, although the results of a simple test made in this connection may be of interest.

*Methods.*—Apple cuttings of current season's growth and of one-year-old and two-year-old wood were used. The basal end of each cutting was treated with a solution of iodine and potassium iodid in water. The cuttings were then divided into two groups, according to whether they contained little or much starch. The treated surfaces were carefully cut away with a sharp knife, and each lot of cuttings was placed in a desiccator over water. The material was sprayed every day with water from an atomizer so as to assure further favorable moisture conditions. Readings were made after 20 days.

*Results.*—Table 16 shows that the difference in starch content of the cuttings was not correlated with any apparent difference in the abundance of callusing.

TABLE 16. *Influence of Starch Content on Amount of Callus*

Age of Wood	Number of Pieces	Average Callus per Individual Cutting					
		Cuttings of Low Starch Content			Cuttings of High Starch Content		
		Lip	Side	Top	Lip	Side	Top
Current season's.....	7	5.0	1.0	0.75	3.0	1.0	1.0
1-year-old.....	11	3.0	1.5	2.0	3.0	1.0	1.0
2-year-old.....	5	3.0	1.0	1.0	2.0	1.0	1.0

*Discussion.*—This bit of evidence cannot be taken with any degree of finality, but it indicates that polarity, as it influences the formation of callus, may not be dependent on a simple food relation; it suggests that the polarity may be based on a complex of factors imperfectly understood at the present time. Kostoff (1928), working with whip grafts of solanaceous plants, found that starch accumulates just above the callus, and stated that "this great accumulation of food is the specific cause of the proliferations." While the dependence of callus development on the accumulation of food is an interesting possibility, it has not been satisfactorily demonstrated as yet, so far as the writer knows.

Since these studies have pertained particularly to root-grafts in which the top of the root-piece is united with the bottom of the scion-piece, nearly



all measurements of root callusing have been of callus formed from the top ends of the root-pieces. Many observations indicate that the root calluses practically as well as the scion (though root callusing may not be so uniform, owing to inherited differences because of seedling origin), but the top end of the root neither calluses as well as the bottom end of the scion nor as well as the bottom end of the root.

*Significance of Downward Polarity in the Formation of Callus Overgrowths*

The preceding experiments have shown the distinct characteristic of apple scion and root cuttings to form callus tissue more abundantly from the lower ends. This fact seems to have a direct relation to the usual occurrence of graft-union overgrowths as proliferations of the scion (particularly the lip) rather than of the root stock, and further supports the view that overgrowths found at the unions of young apple trees are frequently of callus derivation, their formation being very largely influenced by this decided polarity. Plate XXII, figure 1 shows Wealthy grafts with overgrowths of scion derivation at the unions as well as those of stock origin at the bottom of the root pieces.

**Effect of Variety on Callus Formation**

Observations on the callusing of cuttings of several apple varieties have repeatedly shown that varietal differences exist both in the rate of forming callus and in the final abundance attained under like external conditions. Since nurserymen find some varieties more susceptible to "callus knot" than others, a demonstrated correlation between varietal callusing capacity and varietal susceptibility to overgrowths may be regarded as of particular interest.

*Experiment 14 (Variety)*

*Methods.*—See experiment 6. Any procedure that permits the normal formation of callus serves to illustrate the importance of variety.

*Results.*—Table 17 shows that different varieties vary markedly in the

TABLE 17. *Comparative Amounts of Callus from Scion Cuttings of Five Varieties*

Variety	Total Callus in Millimeters		
	7 Days	16 Days	21 Days
Yellow Transparent.....	22.25	63.75	78.50
Wolf River.....	27.50	63.00	71.25
Ben Davis.....	24.25	61.50	62.75
Delicious.....	15.25	34.50	37.75
Willow Twig.....	10.00	35.50	49.75

rate at which they form callus. These data indicate that Yellow Transparent, Wolf River, and Ben Davis are rapid callusing varieties, whereas the Delicious and Willow Twig are comparatively slow callusing varieties.

Other observations have indicated that in general the varieties that

exhibit the highest initial rates of callus formation are also the ones that develop the largest final volumes before callus growth ceases. The Yellow Transparent was always found to be a prolific callusing variety. In many experiments this variety was used in conjunction with the Wealthy and Jonathan varieties, and in these experiments the Yellow Transparent usually produced the most callus, the Wealthy less, and the Jonathan least. This is shown in tables 18 and 19. Differences between varieties in abun-

TABLE 18. *Comparative Amounts of Callus from Cuttings and Grafts of Three Varieties*

Variety	Total Callus in Millimeters	
	21 Days	52 Days
Yellow Transparent.....	70.0	132.0
Wealthy.....	66.0	126.0
Jonathan.....	58.0	112.7

TABLE 19. *Comparative Amounts of Callus from Cuttings and Grafts of Two Varieties*

Variety	Total Callus in Millimeters	
	18 Days	50 Days
Yellow Transparent.....	76.0	141.0
Jonathan.....	52.0	114.0

dance of callusing are more marked in some instances than in others, but consistent differences usually exist.

*Discussion.*—It is a very significant fact that the abundant-callusing varieties have generally been found to be particularly subject to union overgrowths. Thus, the Yellow Transparent and Wealthy varieties knot badly as compared with many other varieties. In a study of overgrowths Muncie (1926) frequently used the Wealthy variety because of the high percentage of trees of this variety which were discarded in the nursery on account of overgrowths at the graft union. If a large number of commercially grown apple varieties were arranged in a series according to the abundance with which their cuttings formed callus under similar conditions, it seems very probable that this series would agree closely with a series based upon susceptibility to callus knot in the field.

#### GENERAL DISCUSSION OF RESULTS

Just as suitable conditions of heat, moisture, and oxygen are essential for the growth of all plants and animals, so are they essential for the growth of callus cells. Published information is very limited with reference to the influence of temperature upon callus development. This is especially true with respect to the callusing of stem and root cuttings of apple. There has been an equal lack of information as to the effects of moisture, as well as of other environmental factors, on callus formation. The present study was begun with a view of gaining more definite information on some of these points. It was thought that it would yield data which, in addition to being of general scientific value, might be useful in apple root-grafting practices, applying not only to the formation of wound tissue but to the prevention of excessive callusing.

The results show that temperature may be employed as an effective instrument in the control of callus formation. Within a wide temperature range, higher temperatures greatly accelerate callus formation. A temperature between 0° and 5° C. lies close to the lower limit of this range, and allows only a small amount of callus to be formed after a period of several months. At 10° C. callus formation is more rapid than at 5°, and at 15° it proceeds at a still higher rate. Growth curves within this range are uniformly concave (indicating an acceleration with the lapse of time), whereas curves for temperatures ranging from 20° upwards are convex (showing rapid growth of callus during the early part of the storage period, but a gradual retardation later). Although the rate of callus formation increases with rise in temperature, if the temperature is above 32° C. pronounced injury occurs. Above this point browning of the surface cells takes place, and the injured cells are readily attacked by molds. By the proper manipulation of the temperature a desired degree of callusing may be had in a given length of time. The temperature can be so regulated that apple grafts can be callused to a point where they are ready for setting out within several days' time, or by using a lower temperature the time for reaching this abundance of callusing may be extended over several months. Or the grafts may be properly callused and then kept at a sufficiently low temperature to prevent further bud, root, and callus development until such a time as they can be planted.

The moisture conditions of the environment have a pronounced retarding influence on callus formation when they are such as to allow considerable desiccation of the tissues. The percentage relative humidity of a surrounding atmosphere seems to be of less importance than the continuous availability of liquid water to the plant material. Cuttings that were exposed to 100 percent relative humidity usually callused only slightly; but if the cuttings were completely covered with a moist medium of peat moss, sphagnum moss, or sand, so that a film of water was held against their surfaces, they callused abundantly. Some callus will form in atmospheres of less than 100 percent relative humidity, but it is usually meager in amount and short-lived. Once saturation of the atmosphere has been attained and a film of liquid moisture has been made available to the plant materials, little or no stimulation of callus development results from increasing the moisture content of the surrounding medium. It was found that with the type of peat moss used, 100 percent water (with respect to dry weight of the peat moss) was sufficient to permit good callusing, and that the water content of the medium may be increased several times (to 300-400 percent) with no great modification of result. Peat moss was found to be a very satisfactory medium for experiments on callusing because of its great absorptive and water-holding capacity. If cuttings or grafts are covered deeply with peat moss containing 300 percent water, no further water need be added under ordinary conditions for a month or more.

Sand is perhaps the most commonly employed medium for callusing of cuttings and grafts, but it differs greatly from peat moss in its physical properties. When water is added to sand the spaces between the particles are flooded (absorption is negligible), but this water is held very loosely due to the coarseness of the medium and to its inability to absorb. In a warm, dry room water must frequently be added to the medium. The alternate flooding and drying of the medium does not favor callus formation, and unsatisfactory results may be obtained.

Oxygen is essential for callus formation, but a lower percentage than that of ordinary air is sufficient. Callus development is not checked by extremely high concentrations of oxygen. Evidence was obtained which indicates that high concentrations of carbon dioxide inhibit callusing.

Apple cuttings, whether shoot or root in derivation, manifest a distinct basal polarity in the formation of callus. That is, the lower end of both scion and root cuttings calluses with distinctly greater abundance than the upper end. If a slanting cut be made on the basal end of a scion cutting, the lower end, or lip, of this cut surface produces far more callus than the upper end. The top end of either a scion or a root cutting calluses comparatively poorly, regardless of whether the cut is transverse or slanting. The explanation of this polarity is not known. It does not appear to be an effect of gravity, unless gravity influences the cuttings before they were severed from the parent tree. The cuttings manifest the same polarity whether stored for callusing in an upright, a horizontal, or an inverted position. Although preliminary tests have not been successful in showing a dependence of polarity upon food storage, this is a possibility. Root knot or callus overgrowths are doubtless related to polarity in callus formation, since in most cases these are outgrowths from the scion lips of root-grafted trees. Since in a tongue-graft union the scion lip occurs on the bottom end and the root lip occurs on the top end of the cutting, it is the scion lip which calluses the more abundantly. With suitable temperature and moisture conditions and a continuous supply of descending elaborated food from the growing tree, it seems very probable that the original callus roll from the scion lip might continue its growth over several years.

Cuttings from selected varieties differed as to the rate and final abundance of callus formation. The Yellow Transparent, Wealthy, Wolf River, and Ben Davis varieties have been found to be abundant callusers, whereas the Jonathan, Delicious, and Willow Twig varieties produce callus less vigorously. These differences may have a direct relation to varietal susceptibility to overgrowths at the graft union, since the abundant-callusing varieties are particularly subject to proliferations of this type.

#### SUMMARY

Recent investigations on the crown gall disease have indicated that a large proportion of the overgrowths on root-grafted apple trees are in the

nature of callus rather than bacterial tumors. Thus the necessity arose for studying the influence of such important environmental conditions as temperature, moisture, and aëration on the callusing of apple cuttings and grafts. Both constant temperatures (ranging from 0° to 40° C.) and variable temperatures were used. Variable moistures were provided in two ways: (1) by varying the humidity of the atmospheres used, and (2) by varying the water content of a peat moss callusing medium. Aëration conditions included atmospheres of different oxygen, nitrogen, and carbon dioxide concentrations. During the course of the study repeated observations have also been made on polarity phenomena and varietal differences which seemed pertinent to the formation of callus overgrowths. The results may be summarized as follows:

1. The complete range of temperatures permitting the formation of callus from apple cuttings (scion or root) and grafts was found to lie between 0° and 40° C. At 3°–5° only a small amount of callus developed during a period of several months. Between 5° and 32° the rate of callus formation increased and the time elapsing before attainment of final volume decreased with rise in temperature. At temperatures above 32° injury usually resulted, and at 40° death of the tissues, accompanied by mold formation, always occurred within the first few days.

2. By the proper regulation of controlled temperatures the callusing processes may be so accelerated or retarded that, within reasonable limits, a desired degree of callus formation may be had within a given length of time. Hence, apple grafts may be callused over a period of several months; they may be similarly callused within a few days, or, after being properly callused, they may be held in good condition for at least several months before planting.

3. For general callusing purposes, temperatures below 20° C. rather than higher have been found most satisfactory.

4. Variable temperatures do not change the general relations. Callusing is accelerated or retarded according to the degree and duration of the temperature.

5. Air moistures below saturation have generally been found to be inhibiting in their effect on callus formation, since below this point desiccation of the tissues occurs. As the moisture content of the air falls, the rate of desiccation increases.

6. Liquid water, present as a film inclosing the cutting, appears to provide the most favorable moisture conditions for bringing about uniform callusing. Such conditions are supplied by moderately moist peat, sphagnum, or sand.

7. Good callusing takes place in a peat moss medium containing 100 percent water by weight. Practically no increase is brought about by raising the water content of the medium, and no perceptible injury or inhibitory effect occurs if the proportion of water to oven-dried peat moss

is increased from three to four times. With a water content beyond this point, however, aëration probably becomes a limiting factor, and callusing is inhibited.

8. Desiccation of callus tissue is accelerated by increase in temperature and decrease in humidity.

9. Proper aëration was found to be important for callusing. The evidence indicates that while some oxygen is required, an amount of oxygen below that of air (20 percent) is sufficient. Callusing took place in high concentrations of oxygen, but was inhibited in 100 percent oxygen. High concentrations of carbon dioxide, particularly with a limited supply of oxygen, prevented callusing.

10. Both scion and root cuttings of apple manifested a distinct polarity in callus formation. Dominance of the bottom end over the top end of cuttings held regardless of the position of the cuttings, whether upright, horizontal, or inverted.

11. Varieties differ both in the rate and abundance with which they form callus. The Yellow Transparent, Wolf River, Wealthy, and Ben Davis are abundant callusing varieties, and the Jonathan, Delicious, and Willow Twig are moderate callusing varieties.

12. The effects of polarity and varietal differences on callus formation of apple cuttings and grafts appear to be significantly related to the occurrence of overgrowths at the union of root-grafted trees.

Great pleasure is taken in acknowledging indebtedness to those who sponsored this study, Doctor William Crocker of the Boyce Thompson Institute, Professor I. E. Melhus of Iowa State College, and Doctor W. C. O'Kane of the Crop Protection Institute. The writer is also deeply grateful to members of the Boyce Thompson Institute staff for repeated suggestions during the study, and particularly to Doctor P. W. Zimmerman, in whose department the work was performed, as well as to Professors S. F. Trelease and R. A. Harper of Columbia University for advice and criticism in the preparation of the manuscript.

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## EXPLANATION OF PLATES

### PLATE XXII

FIG. 1. Apple grafts (Wealthy × French crab) after growing 86 days in the greenhouse. The graft on the left shows a slight overgrowth from the scion lip, and both grafts show distinct overgrowths from the bottom ends of the root pieces.

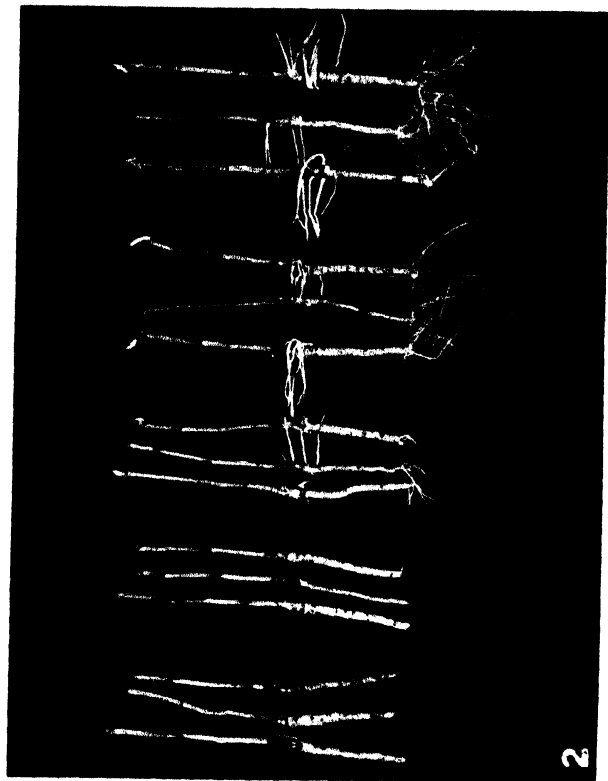
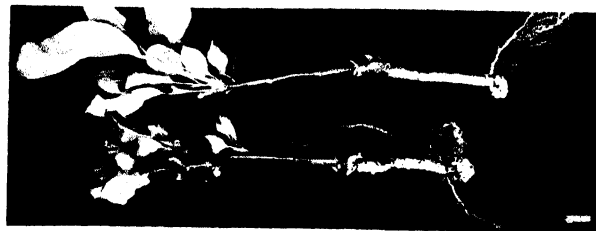
FIG. 2. Apple grafts (Wealthy × French crab) which have been stored in moist peat moss at different temperatures. Typical shoot and root development is shown for different storage temperatures and times. The callusing of the grafts has proceeded nearly parallel with these other growth activities. Reading from left to right: 5° C. for 48 days, 10° for 40 days, 10° for 48 days, 15° for 40 days, and 15° for 48 days.

FIG. 3. Abundant callus formation from the bottom ends of apple cuttings, as follows: upper, Delicious cuttings; lower, French crab root cuttings. The calluses in both cases have grown together.

### PLATE XXIII

FIGS. 1-5. Jonathan cuttings which were callused at similar temperatures in peat moss which varied greatly in water content. The several figures combined illustrate the broad moisture tolerance manifested by callusing tissues. Average moisture contents of the peat moss media (percentage water by weight per 100 parts oven-dried peat moss) for the entire storage period were as follows: reading left to right: 97, 177, 271, 337, and 437 percent. In addition, the effect of polarity on callusing is shown by the unequal ability of the two ends of the same cutting to form callus.

FIG. 6. The figure shows the injury which resulted in high concentrations of oxygen when Wealthy cuttings were stored for 34 days in various mixtures of oxygen and nitrogen. Since callusing of the cuttings was negligible during exposure to the gas, they were transferred to optimum conditions (moist peat at a moderate temperature) in order to test injury. The result is shown after 14 days. The initial gas mixtures for the different sets of three cuttings each were as follows, reading right to left: air check, 0 percent oxygen (nitrogen used as complementary gas in each case), 10 percent oxygen, 20, 30, 40, 50, 60, 70, 80, 90, and 100 percent.



SHIPPY: CALLUS





## THE FUNGICIDAL ACTION OF SULPHUR: I. THE ALLEGED RÔLE OF PENTATHIONIC ACID<sup>1, 2</sup>

FRANK WILCOXON AND S. E. A. MCCALLAN<sup>3</sup>

### INTRODUCTION

Although sulphur and certain of its compounds have been used for many years as a means of combating fungous diseases of plants, it is remarkable that there exists today no uniformity of opinion as to how its fungicidal action is exerted; and there is scarcely a compound of sulphur which might conceivably be formed from the element, under the conditions of use, to which its toxic action has not been attributed. Among these compounds are sulphur dioxide, hydrogen sulphide, sulphuric acid, thiosulphuric acid, and pentathionic acid. Some investigators maintain that the element itself is the active agent, either in finely divided form or as vapor, although its insolubility and low vapor pressure have led the majority to consider it as a source of some more toxic principle. Extensive reviews of the earlier work on this topic are given by Windisch (54), Barker, Gimingham and Wiltshire (2), Doran (12), Young (55), Vogt (48), Thatcher and Streeter (46), Goodwin and Martin (20, 21), and others. There is a great diversity of opinion expressed, and much of the early work is inconclusive and not supported by adequate experimental evidence.

The present paper presents the results of an experimental test of the hypothesis of Young (55), first set forth in 1922, that traces of pentathionic acid associated with sulphur and formed from it constitute the active fungicidal agent. It is hoped to follow this with a second communication dealing with hydrogen sulphide, which has recently become once more of interest through the work of Marsh (30).

It has been shown by Freundlich and Scholz (19), that certain types of colloidal sulphur are stabilized by adsorbed traces of pentathionic acid. These types, called hydrophilic because of the greater degree of hydration of the particles, were found by Young (55, p. 413) to be more toxic than the hydrophobic types which do not contain pentathionic acid. Later he and Williams (51, 52, 56, 57) were able to correlate the toxicity of sulphur dusts with the presence or absence of pentathionic acid on the sulphur par-

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ticles. Several of the published statements of Young in regard to the chemistry of pentathionic acid are at variance with previous chemical knowledge. For example, Young (55, p. 426) states that pentathionic acid is stable only within a narrow range of pH 4.2–5.4; whereas, it is well known that the acid is destroyed by alkalies, and rendered more stable by acids, and does not exhibit any such zone of optimum stability. Thus Freundlich and Schölz (19, p. 265), in discussing the stability of colloidal sulphur, say: "Säuren flocken deswegen so schwach, weil sie die Beständigkeit der Pentathionsäure erhöhen." Again, Young in summarizing the results of his first paper (55, p. 432) states that pentathionic acid is volatile. This is contrary to the impression of previous workers in the field of the polythionic acids, and has not been shown, so far as the present authors are aware. Young does not describe in detail his method of preparing solutions of pentathionic acid, although he and Williams (52, 57) and Williams, Liming and Young (53) give the results of spore germination tests with such solutions, and it is not clear that they ever studied pure solutions of this acid, uncontaminated by other substances. The importance of doing this will be realized when it is remembered that pentathionic acid readily reacts with many other substances forming finely divided sulphur as a product, which would complicate the interpretation of the results. The conclusions of Young have been criticized by Barker (39, p. 313; 1, p. 80) on the ground that sulphur exhibits toxicity outside the limits of pH specified by Young for the existence of pentathionic acid. Goodwin and Martin (20, p. 626) have also questioned the statements of Young regarding the volatility of pentathionic acid. Roach and Glynne (38), in their studies on the winter sporangia of *Synchytrium endobioticum* (Schilb.) Perc., were unable to find any difference in toxicity between pentathionic and sulphuric acids, at the same hydrogen-ion concentration.

In making comparisons of the toxicity of chemical substances to fungus spores, there are two requisites for obtaining accurate results which, though quite obvious, have not always received the consideration they deserve. (a) The substances whose toxicity is to be measured must be available in a pure state and of known concentration, and (b) the technique employed must be capable of distinguishing between the toxicity of the substances it is desired to compare.

#### EXPERIMENTAL METHODS

In these studies spore-germination tests have been employed to determine toxicity and the methods followed are those described in detail by McCallan (31, 32). These are moist-chamber tests and consist essentially of germinating the spores on glass slides in inverted moist chambers. The spores are suspended in the liquid toxic agent and pipetted as drops onto the

slides. In the case of toxic dusts, the slides are first dusted and then an aqueous suspension of spores pipetted onto the slides. The slides are supported on glass racks in the moist chamber, there being four slides to each chamber. Four drops are placed on each slide, hence there are a total of sixteen drops in each chamber. For an illustration of a moist chamber completely set up see McCallan (31). The chambers are sealed with water to preserve an atmosphere of high humidity. These chambers are placed at the desired temperature and the spores examined for germination after a given time. The percentage of germination and average length of germ tubes are recorded.

Redistilled water has been used as the medium for all tests and the temperature in all cases was within the range 19–23° C. The concentration of spores in the drops ranged from about 10 to 40 per low-power field of 1920  $\mu$  diameter. In determining the toxicity of a given compound to a given fungus, the number of spores per field was always approximately the same. The time allowed for germination before examination was from 18 to 48 hours. Since, in none of the fungus spores employed has the percentage of germination appreciably increased after 12 hours, the longer periods of time have merely facilitated greater growth of the germ tubes already formed. In most cases there was but little further elongation after 24 hours.

Four common and representative pathogenic fungi were selected for this study: *Sclerotinia americana* (Wormald) Norton and Ezekiel (13), *Botrytis* sp., of *cinerea* type, *Macrosporium sarcinaeforme* Cav., and *Uromyces caryophyllinus* (Schrank) Winter. The first three species were among those studied by Young in his first paper (55), provided that his *Sclerotinia* was also the common American brown-rot fungus. Young employed *Sclerotinia* almost exclusively in his latter studies. The *Sclerotinia americana* was isolated from infected white sweet cherries at Mattituck, New York, June, 1929. This fungus has been grown on potato-dextrose agar, where it sporulated abundantly. The spore-germination factors for *S. americana* have been discussed by McCallan (32). Since age is important, only conidia from cultures 5 to 10 days old have been used. The optimum germination temperature for these conidia is 23° C. The *Botrytis* sp., was obtained from H. H. Whetzel, Cornell University, who considers it of the *cinerea* type and designates it No. 885 in his collection. The fungus was isolated in 1927 at St. Catharines, Ontario, Canada, from marigold, and has also been grown on potato-dextrose agar, where it sporulates fairly well. The optimum germination temperature was found to be 20°–25° C. The *Macrosporium sarcinaeforme* was isolated by J. G. Horsfall at the Cornell University Experiment Station from red clover in July, 1927. This fungus was likewise grown on potato-dextrose agar and, having a wide temperature

range, the conidia germinated readily at the temperatures employed. The *Uromyces caryophyllinus* was obtained from naturally infected carnation plants of the Early Dawn variety grown in the greenhouses at this Institute. The *Uromyces* uredospores, as is typical of many rust fungi, do not germinate well in the centre of the drop, therefore, germination counts were confined to those in the peripheral zone of the drop. Doran's (11) narrow optimum temperature of 14° C., for the germination of these spores was not substantiated, for it was found that they germinated equally well over the range 10°, 15°, and 20° C., the latter of which was employed in these studies.

The spores from these four fungi are especially suitable because of the representative range of sulphur sensitivity each exhibits. Table 1, compiled from a number of experiments, demonstrates this varying degree of sensitivity to 300-mesh dusting sulphur.

TABLE 1.—The degree of sensitivity to 300-mesh dusting sulphur exhibited by the spores of the fungi employed

Fungus	Spore form	Percentage of germination		Length of germ tubes (μ)	
		Control	Sulphur	Control	Sulphur
<i>Botrytis</i> sp. (cinerea type)...	conidia	99.0	99.0	200	200
<i>Macrosporium sarcinaeforme</i>	conidia	99.0	99.0	400	200
<i>Sclerotinia americana</i> .....	conidia	98.1	60.2	400	80
<i>Uromyces caryophyllinus</i> .....	uredospores	84.2	11.3	400	40

Under field conditions conidia of *Sclerotinia americana* are considered very sulphur-sensitive. It is therefore probable that only those spores whose germ tubes approach the vigor and length of the control would be capable of causing infection.

Much of the published work on spore-germination toxicity tests must be discredited for two reasons: first, because of the lack of sufficiently controlled factors, and secondly, because of the lack of sufficient replications and number of spores counted. In another paper (31) the importance of controlled factors has been stressed. A thorough knowledge of the conditions affecting the germination of the fungus spores to be employed is the first prerequisite. The controls must give consistent germination if any reliance is to be placed on the results obtained. In the studies with *Botrytis* and *Macrosporium* a control germination between 97.5 and 100 per cent was always obtained. In the case of *Sclerotinia americana* seventy-five per cent

of all controls gave a germination between 96.5 and 100 per cent. When the germination percentage in the controls is unusually low a disproportionate effect is obtained with the treated spores, the percentage of germination in the latter being greatly reduced. For this reason, all experiments with *Sclerotinia americana* have been discarded in which the controls have given less than 96 per cent germination. The uredospores of the obligate parasite *Uromyces caryophyllinus* are more variable in their germination.

All experiments have been performed using duplicate and, in some cases, triplicate and quadruplicate moist-chamber tests. In general, but one microscopic field has been counted in each spore-suspension drop; however, in the case of low spore-suspension concentrations more fields have been counted. From 600 to 2000 spores have been counted from the eight slides constituting a duplicate moist-chamber experiment. The larger the number of spores counted the smaller can be the significant difference between treatments. In any experiment of this kind, it is necessary to balance the time and labor involved in counting a large number of spores against the precision attainable in the final result and effect a compromise which will give the desired precision.

From the data obtained in 250 representative duplicate tests, the standard deviation of a single moist-chamber test was calculated by the method of Fleisch (14), and that of the difference between two such tests by the usual formula  $\sigma_{A-B}^2 = \sigma_A^2 + \sigma_B^2$ . The significance of any observed difference between two treatments may then be estimated by referring to a table of values of the probability integral. Unless the difference is at least twice its standard deviation it can not be considered very significant. These values, calculated for five different germination-percentage classes, are shown in table 2.

TABLE 2.—The average percentage of deviation of representative duplicate tests from their mean, and the difference in germination percentage required to show a significant difference between treatments, calculated for five germination-percentage classes

Germination—percentage class	Total number duplicate tests	Average percentage of deviation of duplicates from their mean	Standard deviation of single test: per cent	Standard deviation of difference between two similar tests: per cent	Germination—percentage difference to give odds of 50-1
0- 20	50	0.99	2.27	3.21	7.45
20- 40	50	2.60	4.80	6.79	15.75
40- 60	50	3.78	7.46	10.55	24.48
60- 80	50	2.76	5.61	7.93	18.50
80-100	50	0.45	1.19	1.68	3.90

It will be observed that the mean deviation of duplicates is least in the 0–20 and 80–100 per cent groups, and greatest in the 40–60 per cent group. Because of the variation in the viability of the spores, a repetition of tests at different times, that is different experiments, will not give such consistent results as duplicates of the same experiment at the same time. A similar effect has been observed by Smith (42, p. 31).

#### THE TOXICITY OF PENTATHIONIC ACID

##### *The chemistry of pentathionic acid*

*Historical.*—Pentathionic acid,  $\text{H}_2\text{S}_5\text{O}_6$ , has been the subject of numerous investigations (17, 18, 28, 29, 41) since its discovery by Wackenroder (49) more than eighty years ago, in the solution obtained by passing hydrogen sulphide and sulphur dioxide into water. The difficulties encountered in preparing the salts of this acid in pure form suitable for analysis led some workers to doubt its existence. Spring (44, 45) in a series of papers, the last of which was published in 1882, presented a critical discussion of previous work and concluded that the alleged pentathionic acid was a solution of sulphur in tetrathionic acid. In 1888 Debus (10) published the results of a very thorough study of the Wackenroder solution in the course of which methods were devised for obtaining several of the salts in pure form, by treatment of the concentrated solution with the acetate of the metal whose salt was desired. It was also shown that the salts could be recrystallized from an acidulated solution with less decomposition than from pure water. The work of Debus eliminated all doubt as to the existence of the acid, and subsequent investigators have dealt with the mode of its formation and decomposition (16, 24, 35, 36), structure, physical properties (15, 23), and its quantitative determination in the presence of other sulphur acids (25, 27, 37). Some of these questions have not yet been satisfactorily settled (3). It was found by Salzer (40) that pentathionic acid was formed in an acidified solution of sodium thiosulphate if a small amount of arsenious acid were present. This method of preparation of the acid and its salts has been used by Raschig (34) and has been thoroughly studied by Kurtenacker and Czernotzky (26). It furnishes a much more convenient method of preparation than the original method of Wackenroder, since, by suitable adjustment of the concentrations of the reactants, the yield of pentathionic acid can be made large and that of other polythionic acids quite small.

In addition to these two methods of preparation it has been shown that pentathionic acid is formed by the hydrolysis of sulphur monochloride (19, p. 266), as well as by leading sulphur vapor and water vapor through a heated tube (22). Of special interest in connection with this investigation

is the suggestion of Brugnattelli and Pelloggio (6) that oxidation of sulphur in the presence of moisture leads to the formation of pentathionic acid as an intermediate product in the formation of sulphuric acid. An interesting case of the occurrence of pentathionates in nature is reported by Day and Allen (9, pp. 115-118). These authors found that salt incrustations around hot springs near Lassen Peak, Shasta County, California, contained considerable amounts of soluble pentathionates.

Pentathionic acid has not been obtained in a pure state, although aqueous solutions containing 50-60 per cent of the acid may be prepared. It is a strong acid comparable to sulphuric acid and may be titrated with methyl-orange indicator. It is fairly stable in the presence of other strong mineral acids, but is at once decomposed by addition of an excess of sodium hydroxide solution, with separation of elementary sulphur in finely divided form, and this reaction forms the basis of a characteristic qualitative test for pentathionic acid, since no other oxygen acid of sulphur gives this test, with the possible exception of the recently prepared hexathionic acid (50). A less conclusive test is the formation of silver sulphide when a solution containing pentathionic acid is treated with ammoniacal silver-nitrate solution.

Among other reactions of pentathionates may be mentioned that with sodium sulphite forming sodium trithionate and thiosulphate, which has been used as a rapid titration method by Kurtenacker and Bittner (25). Hydrogen sulphide also rapidly decomposes pentathionic acid forming sulphur and water, according to Debus (10).

*Preparation of potassium pentathionate and barium pentathionate.*—In order to test the hypothesis that pentathionic acid is a factor in the toxic action of sulphur on fungus spores, it is desirable to prepare solutions of the acid from its purified salts, since the crude preparations obtained by the method of Wackenroder or of Salzer may contain a number of other compounds in addition to pentathionic acid. The potassium salt has been prepared by a number of investigators and its optical and crystallographic properties described by Fock and Klüss (15). Barium pentathionate has been prepared by Lenoir (29) and by Fordos and Gélis (18), and these authors note that the barium salt when precipitated by alcohol persistently retains small amounts of alcohol.

The procedure used in this investigation was the same as that described by Raschig (34, pp. 274-289). The final syrupy solution of pentathionic acid was freed from sodium pentathionate as described by him and treated with potassium acetate and acetic acid in alcohol. The potassium salt was recrystallized once from water containing 1 per cent sulphuric acid, washed with alcohol, and dried over calcium chloride. In the case of the barium salt, a concentrated solution of barium acetate, containing 3 per cent of free acetic acid was added to the pentathionic acid and barium sulphate filtered



off. On treating the filtrate with an equal volume of alcohol, the barium pentathionate separated in well-formed rectangular tablets. This salt was dissolved in water acidulated with acetic acid, and reprecipitated with alcohol. The proportion of potassium or of barium in these preparations was determined by ignition to constant weight with an excess of concentrated sulphuric acid and that of sulphur by oxidation with bromine and hydrochloric acid followed by precipitation as barium sulphate.

TABLE 3.—*Results of analyses of potassium pentathionate and barium pentathionate*

Potassium pentathionate			Barium pentathionate		
	Found	Calculated for $K_2S_5O_{10} \cdot 1\frac{1}{2} H_2O$		Found	Calculated for $BaS_5O_{10} \cdot 3\frac{1}{2} H_2O$
Potassium ...	21.67%	21.63%	Barium .....	30.21%	30.08%
Sulphur .....	44.16%	44.34%	Sulphur .....	35.30%	35.10%
Ratio K-S...	2-4.97	2-5	Ratio Ba-S...	1-5.01	1-5

The crystals dissolved in water, formed a clear solution and gave the qualitative tests for a pentathionate, *i.e.*, an immediate precipitation of sulphur with sodium hydroxide and a brownish black precipitate with ammoniacal silver nitrate. A 0.1-gram portion in 10 c.c. did not decolorize 1 drop N/10 iodine, indicating the absence of sulphites and thiosulphates, and gave no precipitate with barium chloride, indicating the absence of sulphates. The crystals (Figs. 1 and 2) were submitted for examination to C. W. Mason, of Cornell University, who reports as follows:

“Potassium pentathionate hemitrihydrate. Orthorhombic, (as described by Groth: *Chemische Krystallographie* 2: 717. Leipzig, 1906). 2V about  $65^\circ$ ,  $v < \rho$ , with the axial plane parallel to 010. Optically negative. Refractive indices by the immersion method:  $\alpha = 1.570$ ;  $\beta = 1.63 \pm$ ;  $\gamma = 1.658$ .”

“Barium pentathionate, hydrated. As received: well-formed rectangular tablets, occasionally with their corners truncated by small bipyramid faces. The tablets are flattened parallel to the axial plane, so that only edge views exhibit interference figures. 2E is large, and the optical character is positive (+). Refractive indices by the immersion method:  $\alpha = 1.620 -$ ;  $\beta = 1.640 -$ ;  $\gamma = 1.670$ .”

“The above tablets, placed in water, become covered by numerous small prismatic crystals. Recrystallized from water, in which the salt is very soluble, imperfect tapering four sided prisms with curved edges, very acute ends, and marked transverse cleavage, are obtained. These exhibit extinction varying from parallel to about  $8^\circ$  as a maximum. Interference figures



FIG. 1. Crystals of potassium pentathionate from aqueous solution by addition of alcohol.

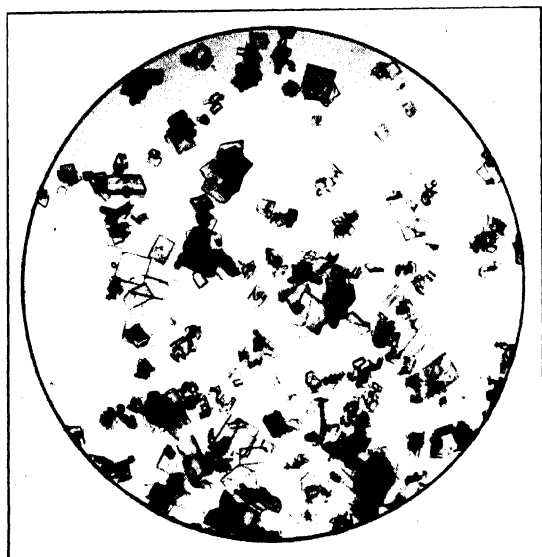


FIG. 2. Crystals of barium pentathionate from aqueous solution by addition of alcohol.

were not obtained on account of the unfavorable habit of the crystals. Approximate refractive indices, by the immersion method:  $\alpha = 1.59$ ;  $\beta = 1.67$ ;  $\gamma = 1.75$ . Recrystallized by the addition of alcohol to a concentrated aqueous solution, crystals like those from water are first formed, singly and in clusters. As more alcohol is added these dissolve and rectangular tablets appear, finally replacing them completely. This indicates the existence of two different degrees of hydration."

Solutions containing pentathionic acid were obtained by treating 1 gram of the potassium salt with the calculated quantity of tartaric acid required to liberate the pentathionic acid and form potassium acid tartrate, if the reaction were complete. It was found, however, by weighing the potassium acid tartrate which crystallized out, that the conversion was approximately 50 per cent complete and the final solution contained pentathionic acid, potassium pentathionate, and tartaric acid.

In the case of the barium salt, solutions of pentathionic acid were obtained by adding to a known solution of the salt the exact quantity of standard sulphuric acid required to precipitate the barium as sulphate. The latter was filtered off and the solution diluted to the concentration desired. The pentathionic acid solutions obtained in this manner were quite stable, did not deposit sulphur on standing, and still gave tests for pentathionic acid after keeping for a month.

*The relative toxicity of pentathionic acid, sulphuric acid, and  
hydrogen sulphide*

In this series of experiments, the comparative toxicity of pentathionic acid, sulphuric acid, and hydrogen sulphide was determined. Sulphuric acid was chosen for comparison because it is a typical strong mineral acid, as is pentathionic acid, but the sulphate ion shows no marked toxicity to fungus spores (7). Sulphuric acid is commonly present in commercial sulphur, and in greater quantities than pentathionic acid, and finally, it was used as a standard of comparison by Young and his coworkers (52, 53). Preliminary results with hydrogen sulphide are included, since the recent work of Marsh (30) appears to confirm the early findings of Polacci (33) that this substance is an important factor in the fungicidal action of sulphur. Hydrogen sulphide rapidly escapes from aqueous solutions exposed to the air. Hence it was necessary to perform the toxicity tests in a closed vessel. The hydrogen-ion concentration of the sulphuric acid and pentathionic acid solutions was determined with the antimony electrode (4, pp. 83, 84), because irregular results were obtained with pentathionic acid when the hydrogen electrode was used. The results of this series of experiments are presented in tables 4 to 8 and figures 3 to 10.

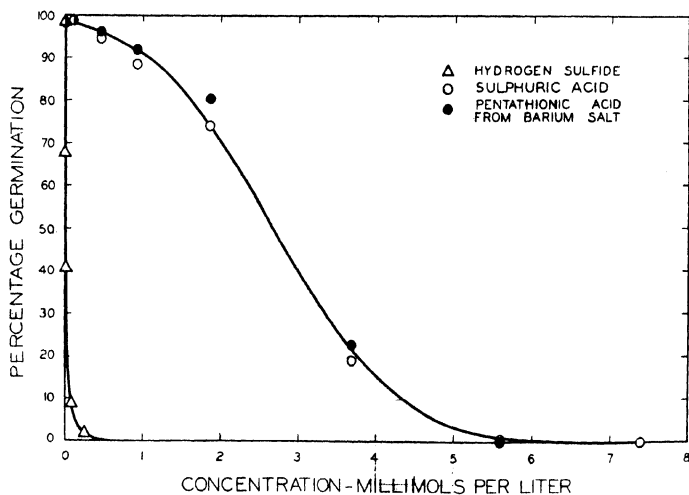


FIG. 3. The percentage of germination of conidia of *Sclerotinia americana* in solutions containing varying concentrations of  $\text{H}_2\text{S}$ ,  $\text{H}_2\text{SO}_4$ , and of  $\text{H}_2\text{S}_5\text{O}_6$  prepared from the barium salt.

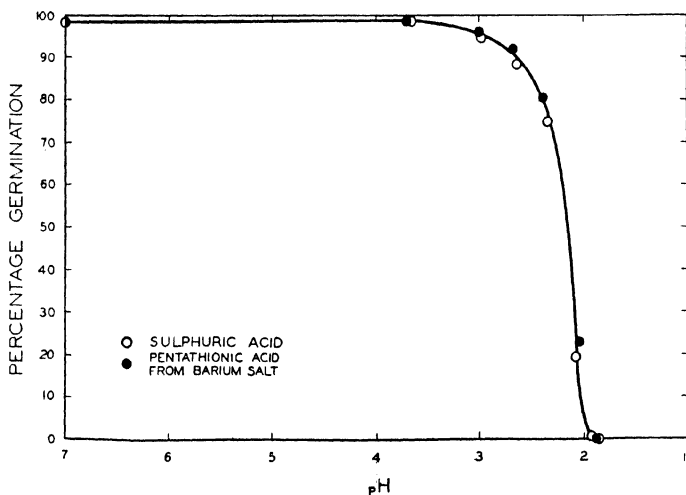


FIG. 4. The percentage of germination of conidia of *Sclerotinia americana* in solutions of  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{S}_5\text{O}_6$ , plotted against the pH of these solutions.

TABLE 4.—The comparative toxicity of pentathionic acid and sulphuric acid solutions to the conidia of *Sclerotinia americana*

Pentathionic acid (from barium salt)				Sulphuric acid			
Concentration (millimols per litre)	pH	Percentage germination	Germ-tube length ( $\mu$ )	Concentration (millimols per litre)	pH	Percentage germination	Germ-tube length ( $\mu$ )
Control	—	98.4	300	Control	—	98.4	300
0.092	3.70	98.6	300	0.092	3.65	98.7	320
0.462	3.00	96.0	250	0.462	2.98	94.7	225
0.930	2.67	91.9	175	0.930	2.64	88.2	150
1.860	2.38	80.3	100	1.860	2.35	74.9	80
3.696	2.04	22.9	40	3.696	2.07	19.3	35
5.604	1.88	0	0	5.604	1.92	0.5	25
—	—	—	—	7.392	1.86	0	0

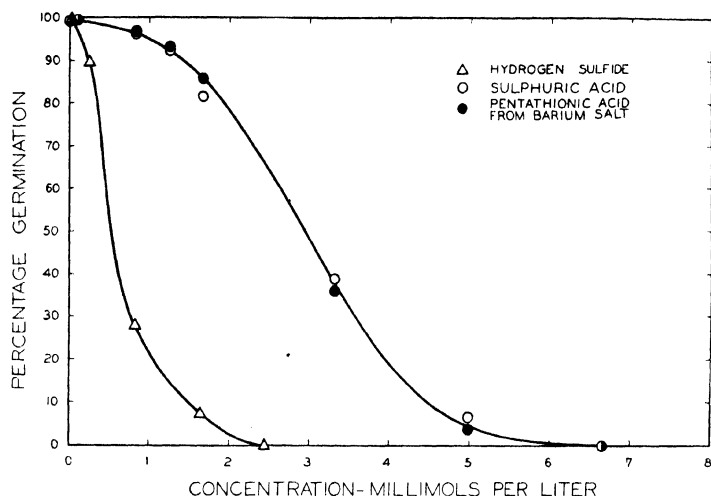


FIG. 5. The percentage of germination of conidia of *Botrytis* sp. (*cinerea* type) in solutions containing varying concentrations of  $H_2S$ ,  $H_2SO_4$ , and of  $H_2S_5O_6$  prepared from the barium salt.

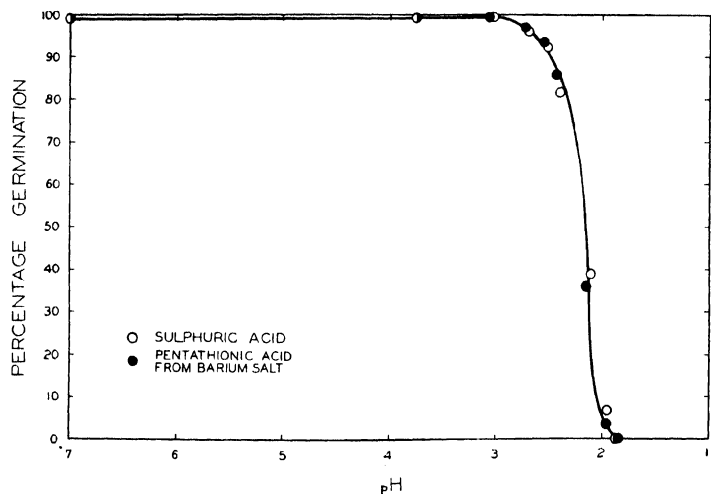


FIG. 6. The percentage of germination of conidia of *Botrytis* sp. (*cinerea* type) in solutions of  $H_2SO_4$  and  $H_2S_5O_6$ , plotted against the pH of these solutions.

TABLE 5.—*The comparative toxicity of pentathionic acid and sulphuric acid solutions to the conidia of Botrytis sp. (cinerea type)*

Pentathionic acid (from barium salt)				Sulphuric acid			
Concentration (millimols per litre)	pH	Percentage of germination	Germ-tube length ( $\mu$ )	Concentration (millimols per litre)	pH	Percentage of germination	Germ-tube length ( $\mu$ )
Control	—	99.0	200	Control	—	99.0	200
0.084	3.75	99.3	200	0.084	3.75	99.3	200
0.418	3.06	99.3	200	0.418	3.03	99.3	200
0.835	2.72	96.8	150	0.835	2.70	96.0	150
1.254	2.54	93.2	100	1.254	2.52	92.2	100
1.669	2.43	85.7	85	1.669	2.40	81.5	75
3.332	2.15	35.9	45	3.332	2.11	38.9	50
4.998	1.95	3.4	25	4.998	1.95	6.3	30
6.664	1.85	0	0	6.664	1.87	0	0

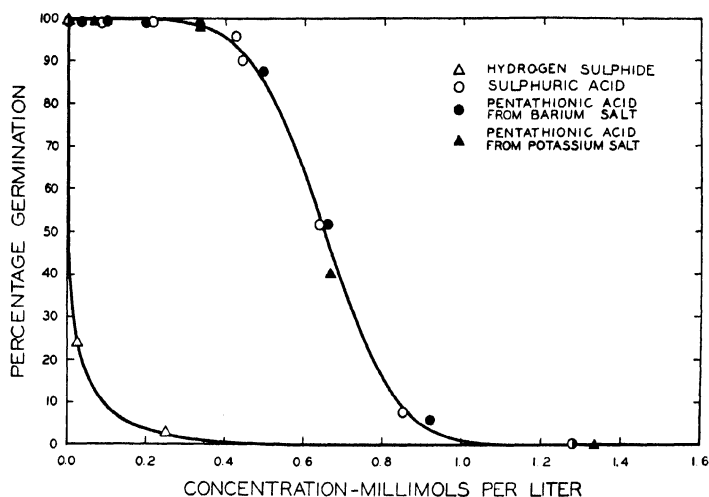


FIG. 7. The percentage of germination of conidia of *Macrosporium sarcinaeforme* in solutions containing varying concentrations of  $\text{H}_2\text{S}$ ,  $\text{H}_2\text{SO}_4$ , and of  $\text{H}_2\text{S}_5\text{O}_6$ , prepared from the barium and potassium salts.

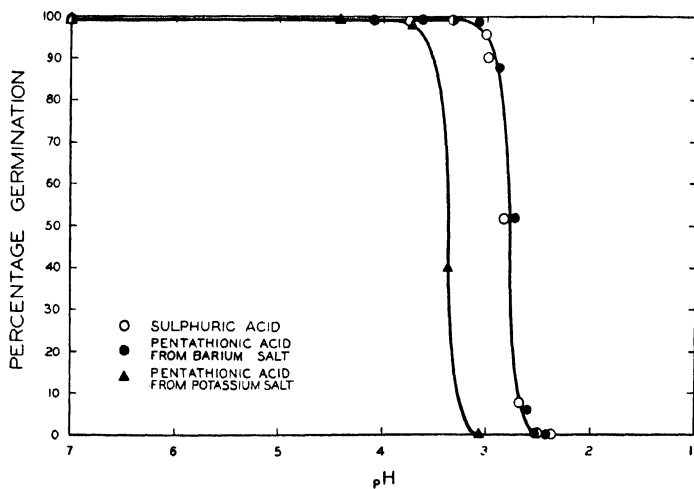


FIG. 8. The percentage of germination of conidia of *Macrosporium sarcinaeforme* in solutions of  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{S}_5\text{O}_6$  plotted against the pH of these solutions.



TABLE 6.—The comparative toxicity of pentathionic acid and sulphuric acid solutions to the conidia of *Macrosporium sarcinaeforme*

Pentathionic acid							Sulphuric acid			
From barium salt				From potassium salt						
Concentration (millimols per liter)	pH	Percent- age of germina- tion	Germ- tube length ( $\mu$ )	Concentration (millimols per liter)	pH	Percent- age of germina- tion	Germ- tube length ( $\mu$ )	Concentration (millimols per liter)	pH	Percent- age of germina- tion
Control	—	99.3	400	Control	—	99.0	400	Control	—	99.3
0.033	4.08	99.1	500	0.006	5.50	99.0	400	0.084	3.75	98.8
0.099	3.62	99.2	500	0.064	4.42	99.0	400	0.214	3.34	99.0
0.199	3.33	99.0	500	0.333	3.73	97.7	250	0.426	3.02	95.7
0.332	3.07	98.7	500	0.667	3.40	40.0	120	0.437	3.00	90.0
0.497	2.88	87.7	300	1.333	3.08	0	0	0.640	2.83	51.6
0.663	2.73	51.8	200	—	—	—	—	0.853	2.68	7.5
0.924	2.60	5.9	30	—	—	—	—	1.280	2.51	0.2
1.280	2.53	0.2	15	—	—	—	—	1.706	2.38	0
1.710	2.42	0	0	—	—	—	—	—	—	—

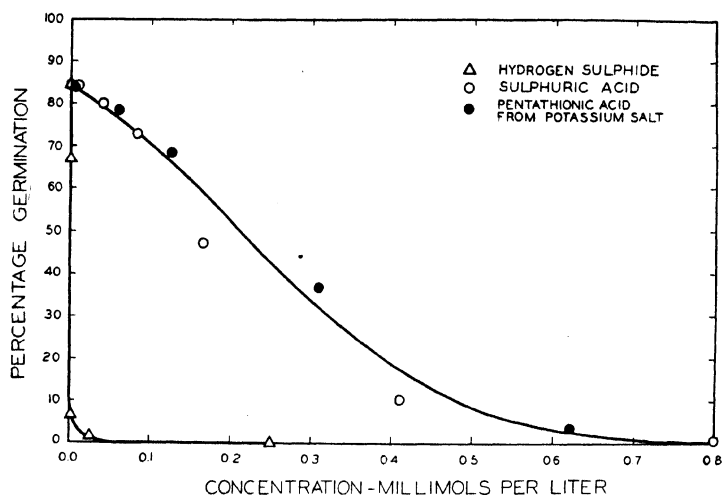


FIG. 9. The percentage of germination of uredospores of *Uromyces caryophyllinus* in solutions containing varying concentrations of  $H_2S$ ,  $H_2SO_4$ , and of  $H_2S_5O_6$  prepared from the potassium salt.

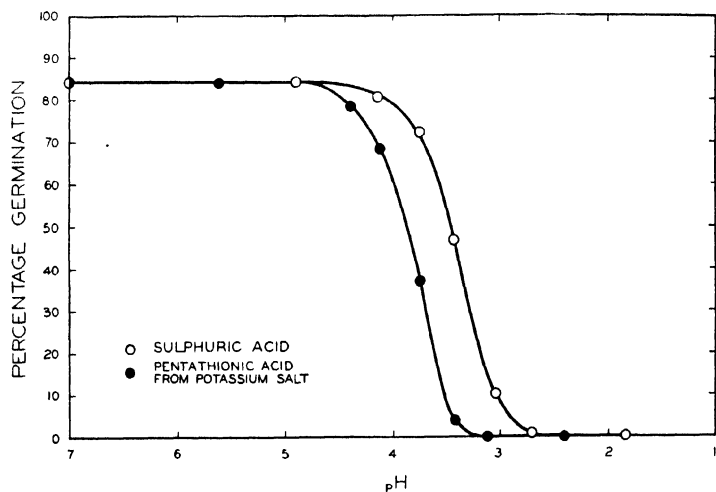


FIG. 10. The percentage of germination of uredospores of *Uromyces caryophyllinus* in solution of  $H_2SO_4$  and  $H_2S_5O_6$ , plotted against the pH of these solutions.

TABLE 7.—*The comparative toxicity of pentathionic acid and sulphuric acid solution of the uredospores of Uromyces caryophyllinus*

Pentathionic acid (from potassium salt)				Sulphuric acid			
Concentration (millimols per litre)	pH	Percentage of germination	Germ-tube length ( $\mu$ )	Concentration (millimols per litre)	pH	Percentage of germination	Germ-tube length ( $\mu$ )
Control	—	84.2	400	Control	—	84.2	400
0.006	5.60	83.8	400	0.008	4.90	84.2	400
0.062	4.40	78.1	300	0.041	4.15	80.0	300
0.124	4.12	68.5	200	0.082	3.75	72.7	200
0.311	3.76	36.9	150	0.163	3.45	46.9	150
0.622	3.43	3.9	50	0.408	3.05	10.1	75
1.245	3.12	0	0	0.816	2.71	0.8	25
6.224	2.42	0	0	8.157	1.84	0	0

Since the results obtained with a given fungus, using sulphuric and pentathionic acids, are in no case significantly different, as was also found by Roach and Glynne (38), working with *Synchytrium endobioticum*, a single curve has been drawn through all the points obtained. This curve was derived by plotting the points on probability paper (47, pp. 263-270) and drawing the best straight line through the points by the method of least squares. The sigmoid curve obtained in this way, which may be considered as the integrated form of a probability curve, appears to fit the data quite well. The theoretical significance of such a curve in relation to toxicity experiments has been discussed by Brooks (5, p. 78) and Smith (42, pp. 31-34; 43, pp. 340-341). It will be observed that in every case hydrogen sulphide exhibits much greater toxicity than either sulphuric acid or pentathionic acid.

When the percentage of germination is plotted against pH for sulphuric and pentathionic acids, no evidence is obtained for an optimum zone of toxicity. The solutions show no effect until a rather high acidity is reached, which varies for the different species of fungi, and the curves then drop sharply, the point of complete inhibition of germination being reached quite rapidly.

The difference in effect of the pentathionic acid solutions made from the potassium salt and those made from the barium salt may perhaps be ascribed to the content of foreign material in the former, namely, potassium pentathionate and tartaric acid, which contribute much to the total molecular concentration but little to the acidity.

An interesting observation frequently made was that discarded solutions of pentathionic acid became contaminated with a species of *Penicillium*, which grew luxuriantly in these solutions. This was not the case with the sulphuric acid solutions.

#### *The toxicity of the salts of pentathionic acid*

Although, according to Williams and Young, free pentathionic acid exhibits marked toxicity, these authors state that the neutral salts are nontoxic (52, p. 361). Accordingly, a study has been made of the toxicity of potassium pentathionate to the conidia of *Sclerotinia americana*, in distilled water, in an acid buffer solution, and in a solution to which sufficient sodium hydroxide was added to cause incipient decomposition of the potassium pentathionate. The buffer used was the phthalate-NaOH buffer of Clark and Lubs (8) pH 4.6 diluted to  $\frac{1}{4}$  the recommended strength. The results are shown in tables 9 and 10.

Potassium pentathionate in distilled water was entirely nontoxic at the concentration employed. When the salt is placed in an acid buffer a certain amount of free pentathionic acid must be formed, but, even under

TABLE 8.—The toxicity of hydrogen sulphide to the conidia of *Botrytis* sp. (cinerea type), *Macrosporium sarcinaeforme*, *Sclerotinia americana* and to the uredospores of *Uromyces caryophyllinus*

Concentration (millimols per litre solution)	<i>Botrytis</i> sp. (cinerea type)		<i>Macrosporium sarcinaeforme</i>		<i>Sclerotinia americana</i>		<i>Uromyces caryophyllinus</i>	
	Percentage of germination	Germ-tube length ( $\mu$ )	Percentage of germination	Germ-tube length ( $\mu$ )	Percentage of germination	Germ-tube length ( $\mu$ )	Percentage of germination	Germ-tube length ( $\mu$ )
Control .....	99.2	200	99.4	400	98.8	400	84.2	400
0.0002 .....	—	—	—	—	—	—	66.8	75
0.0025 .....	—	—	—	—	67.4	150	6.4	30
0.0250 .....	99.4	225	24.1	100	40.5	100	1.3	15
0.0840 .....	—	—	—	—	8.5	50	—	—
0.2500 .....	89.5	175	2.6	50	1.5	25	0	0
0.4600 .....	—	—	—	—	—	—	—	—
0.8300 .....	27.8	75	—	—	—	—	—	—
1.6500 .....	7.2	50	—	—	—	—	—	—
2.4600 .....	0.1	15	0	0	—	—	0	0

TABLE 9.—*The toxicity of potassium pentathionate solutions to the conidia of Sclerotinia americana*

Solution	Percentage of germination	Germ-tube length ( $\mu$ )
Control .....	99.2	500
0.10% $K_2S_2O_8 \cdot 1\frac{1}{2}$ H <sub>2</sub> O in redistilled water .....	99.4	500
0.01% $K_2S_2O_8 \cdot 1\frac{1}{2}$ H <sub>2</sub> O in potassium acid phthalate— NaOH buffer, pH 4.6 .....	99.4	500
0.10% $K_2S_2O_8 \cdot 1\frac{1}{2}$ H <sub>2</sub> O in potassium acid phthalate— NaOH buffer, pH 4.6 .....	99.1	500
0.50% $K_2S_2O_8 \cdot 1\frac{1}{2}$ H <sub>2</sub> O in potassium acid phthalate— NaOH buffer, pH 4.6 .....	99.2	500
Potassium acid phthalate—NaOH buffer, pH 4.6 .....	99.4	500

TABLE 10.—*The toxicity, to the conidia of Sclerotinia americana, of colloidal sulphur formed from solutions of potassium pentathionate and sodium hydroxide*

Solution	pH	Percentage of germination	Germ-tube length ( $\mu$ )
Control .....	—	98.5	275
95 c.c. 0.5% $K_2S_2O_8 \cdot 1\frac{1}{2}$ H <sub>2</sub> O + 5 c.c. N/10 NaOH .....	6.72	0	0
ditto, diluted 1-10 .....	6.40	62.5	75
5 c.c. N/10 in 100 c.c. redistilled H <sub>2</sub> O .....	11.14	83.9	200
ditto, diluted 1-10 .....	10.49	97.6	350

these conditions, no toxicity was exhibited, although the pH value of 4.6 lies within the range of maximum toxicity according to Young (55, p. 410).

In the case of the solutions to which sodium hydroxide was added, due to partial decomposition, colloidal sulphur was formed, and these solutions were highly toxic.

#### THE TOXICITY OF WATER EXTRACTS FROM SULPHUR

Williams and Young (52, p. 359) have stated that pentathionic acid is found in filtered water extracts from sulphur. In another place (57, p. 19) the same authors say that this acid is adsorbed quite completely by the sulphur particle so that none can be washed off. According to our experience most samples of sulphur give water extracts which respond to tests given by pentathionic acid. (a) They form a brownish black precipitate of silver sulphide with ammoniacal silver nitrate. (b) When hydrogen sulphide is passed through the extracts for several minutes, colloidal sulphur is formed. (c) There is a slight precipitate formed when the extracts are made alkaline with sodium hydroxide. None of the extracts

examined gave the methylene blue test for hydrogen sulphide. The reactions obtained were, however, not very distinct, and other substances than pentathionic acid might have given each of them; we are only justified in saying, therefore, that there is a strong probability of the existence of traces of pentathionic acid in the water extracts but not an absolute certainty.

The toxicity of water extracts from several samples of sulphur has been determined. These extracts were made by triturating 100 gram portions of sulphur in a mortar with 100 c.c. of distilled water and filtering the mixture. Three of the sulphur samples were obtained from a well-known firm dealing in chemical reagents and correspond to the sulphur preparations listed in the U. S. Pharmacopeia and others were two well-known brands of commercial 300-mesh dusting sulphur. One of the latter gave an alkaline water extract which showed the presence of calcium when tested with ammonia and ammonium oxalate. None of the extracts tested showed any toxicity to conidia of *Sclerotinia americana* when prepared as described above. The results appear in table 11.

TABLE 11.—*The toxicity of water extracts from various kinds of sulphur to the conidia of Sclerotinia americana*

Solution	pH	Percentage of germination	Germ-tube length ( $\mu$ )
Control .....	—	99.3	900
Water extract from Sulphur Lotum .....	6.0	99.5	900
“ “ “ Sulphur Praecipitum .....	6.4	99.4	800
“ “ “ Roll Sulphur .....	6.2	99.3	900
Control .....	—	97.6	175
Water extract from commercial dusting sulphur (A) .....	4.2	98.2	180
Water extract from commercial dusting sulphur (B) .....	8.6	98.8	225

THE TOXICITY OF SULPHUR DUST BEFORE AND AFTER  
TREATMENT TO REMOVE ACIDS

When it is desired to compare two samples of sulphur and to determine the effect of some factor on their toxicity, as, for example, the presence or absence of pentathionic acid, it is necessary that the samples should be alike in other respects. One factor that might be expected to influence the toxicity is the particle size of the material. Roll sulphur was ground and sieved to furnish four samples whose particles varied from 33 to 285  $\mu$  in diameter. The average diameter for each lot was determined by micro-

metric measurement of the particles. The toxicity to conidia of *Sclerotinia americana* was determined in quadruplicate tests on each sample. The results are shown in table 12.

TABLE 12.—*The relation between the particle size and toxicity of a sulphur dust to the conidia of Sclerotinia americana*

Treatment	Mean diameter of particle ( $\mu$ )	Percentage of germination
Control .....	—	97.6
Ground Roll Sulphur .....	285	62.8
“ “ “ .....	142	47.2
“ “ “ .....	60	29.1
“ “ “ .....	33	20.7

It will be seen that the toxicity increases to a marked degree as the particles decrease in size, and therefore particle size must be considered in the evaluation of sulphur dusts as fungicides.

If the toxic factor of sulphur were pentathionic acid, it would be reasonable to expect a difference in toxicity between sulphur, treated in such a way as to destroy any traces which might exist, and the original sample. Accordingly, a sample of 300-mesh dusting sulphur which gave a marked test with ammoniacal silver nitrate, was divided into two portions. One of these was triturated in a mortar with one per cent sodium hydroxide solution and allowed to remain in contact with the solution overnight. The next morning it was filtered with suction and thoroughly washed until the washings were neutral to litmus. Just before use, a portion was extracted with distilled water and the extract tested for pentathionic acid with ammoniacal silver nitrate. None was found. Comparative toxicity tests, using the conidia of *Sclerotinia americana*, were then made on the two samples in triplicate, 5000 spores being counted. The results of this experiment are shown in table 13.

TABLE 13.—*The comparative toxicity, to Sclerotinia americana conidia, of sulphur dust before and after treatment to remove pentathionic acid*

Treatment	Percentage of germination	Germ-tube length ( $\mu$ )
Control .....	98.0	400
300-mesh sulphur untreated; with original pentathionic acid content .....	64.7	100
300-mesh sulphur treated to remove content of pentathionic acid .....	64.4	100

There is no significant difference in toxicity between sulphur treated to remove pentathionic acid and untreated sulphur.



## SUMMARY

1. The various theories that have been advanced to account for the fungicidal action of sulphur have been briefly reviewed, with especial emphasis on the pentathionic acid hypothesis of Young.

2. An improved technique has been employed for the laboratory determination of fungicidal activity, by means of spore-germination tests.

3. The spores of four representative pathogenic fungi were used, namely, *Botrytis* sp. (*cinerea* type), *Macrosporium sarcinaeforme*, *Sclerotinia americana*, and *Uromyces caryophyllinus*; these exhibit varying degrees of sulphur sensitivity.

4. The accuracy attained in these tests has been determined, and defined in terms of percentage of germination and odds of significance.

5. The chemistry of pentathionic acid has been discussed, and the preparation, analysis, and properties of potassium and barium penathionates have been described. Pentathionic acid solutions have been prepared from these salts.

6. The comparative toxicity of pentathionic acid, sulphuric acid, and hydrogen sulphide to the four fungi has been determined. It has been found that pentathionic and sulphuric acids, both typical strong mineral acids, exhibit identical toxicity within the error of the experiment. The toxicity of these acids is apparently due to the hydrogen ion, and a comparatively high concentration is required for its manifestation. Hydrogen sulphide is from 6 to 200 times as toxic.

7. When percentage of germination is plotted against concentration of a toxic agent, a sigmoid curve is obtained. This curve appears to be the integrated form of a normal distribution curve, which perhaps indicates the distribution of resistance among the individual spores used.

8. The neutral salts of pentathionic acid were found to be nontoxic to conidia of *Sclerotinia americana*, except when treated with sodium hydroxide, which destroys the pentathionic and forms colloidal sulphur.

9. It has been found that most samples of sulphur give water extracts that respond to qualitative tests for pentathionic acid, but these extracts were not toxic under the conditions of experiment.

10. The particle size of sulphur dusts is an important factor in their toxicity and must be considered in comparing one preparation with another. The toxicity increases with the fineness of subdivision.

11. A commercial 300-mesh dusting sulphur, treated with sodium hydroxide to remove pentathionic and sulphuric acids, did not differ in toxicity from the same preparation before treatment.

12. It is therefore concluded that pentathionic acid is not a factor of importance in the fungicidal action of sulphur.

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## ENZYM ACTIVITIES OF JUICES FROM POTATOES TREATED WITH CHEMICALS THAT BREAK THE REST PERIOD <sup>1, 2</sup>

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### INTRODUCTION

When freshly-harvested potatoes are treated with chemicals such as ethylene chlorhydrin, sodium thiocyanate, and thiourea the rest period is broken, and sprouts begin to make their appearance uniformly throughout the lot, after about five to eight days. The untreated potatoes planted at the same time, however, usually do not show sprouts until several weeks later, and even then the sprouting is not uniform (4).

It seemed desirable to study the enzym changes that take place in the potatoes during these few days in which the processes correlated with the breaking of dormancy are in progress, and to compare them with untreated tubers under the same conditions. This paper presents the results of a series of measurements of the enzym activities of juices obtained from potatoes which had been treated with chemicals but which had not yet sprouted.

The three chemicals used for treating the potatoes were ethylene chlorhydrin ( $\text{ClCH}_2\text{CH}_2\text{OH}$ ), sodium thiocyanate ( $\text{NaSCN}$ ), and thiourea ( $\text{NH}_2\text{CSNH}_2$ ). These, although quite unlike in chemical character, produce similar results in the breaking of dormancy and therefore it seemed of special interest to compare them with regard to their effects upon enzym activity.

The object of the experiment was to obtain partial or complete answers to such questions as the following: What enzymes show the greatest changes? How soon after treatment do the changes start? Do the different chemicals produce the same or different effects upon the enzymes? Is there any relation between the concentration of chemical used in treating the potato and the enzym activity of the press-juice? Is there any correlation between the sprouting response and the enzym changes? Are the chemicals acting directly upon the enzymes or do the chemicals act first upon the living matter and only indirectly upon the enzymes? Do the changes occur in the absence of the eyes or must these be present to permit a response?

The enzymes mainly studied in this series of experiments were catalase,

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peroxidase, and oxidoreductase; a few tests with amylase and invertase were made; and in the course of the tests it was found that the pH changes and certain non-enzymic reducing properties of the juice had to be taken into account.

A general statement of the results is as follows: Increases in various enzymes were induced by the treatments and in some cases these were detectable within 24 hours. The amount of the increase was related to the concentration of the chemical used, in such a manner that a series of chemical treatments of graded strength gave a corresponding gradation of enzyme activities. The three different chemicals were not equal in their effect upon enzymes, ethylene chlorhydrin being much more effective than sodium thiocyanate and thiourea. The correlation between the sprouting produced by the treatment and the amount of increase in enzyme activity was not close, the thiourea treatments, particularly, producing better sprouting than would be expected from the effect upon enzymes. Presence of eyes in seed-pieces treated with chemicals was not necessary for increases in enzyme activity. Increases could not be obtained by adding the chemicals to the press-juice; they occurred only when the potatoes were treated with chemicals and press-juices obtained at a subsequent period.

## MATERIALS AND METHODS

### Varieties and Source of Seed

Tubers of both Irish Cobbler and Bliss Triumph varieties were used. Freshly harvested potatoes of Irish Cobbler were obtained early in June from Mr. C. E. McLeod, Jr., of Seabrook, S. C., late in June from Dr. R. A. McGinty of Clemson College, S. C., and in mid-July from Mr. J. M. Snyder of College Park, Maryland. To these persons and to Dr. C. O. Appleman of the University of Maryland the writers wish to express thanks for their valuable coöperation in obtaining a dependable supply of experimental potatoes. For the tests later in the season tubers of Bliss Triumph and Irish Cobbler harvested in August from the Institute gardens were used. The Bliss Triumph seed potatoes from which this crop was grown were kindly furnished by the Nebraska Certified Potato Growers Association through the courtesy of Mr. William Morrow and Prof. H. O. Werner of the University of Nebraska, to both of whom we wish to make grateful acknowledgment. The response of tubers of these different varieties from different localities was not noticeably dissimilar so far as the enzyme studies were concerned. Bliss Triumph has a shorter dormant period than Irish Cobbler and responds somewhat more readily to chemical treatment but the changes observed were always in the same direction and approximately equal in amount in the various lots. A much greater divergence was found between the results with whole tubers and cut tubers of the same variety than between varieties.

### Chemical Treatments

The methods used in treating the potatoes were the same as those described in previous publications (4, 5). The ethylene chlorhydrin "dip method" consisted in dipping the cut tubers (one-eye pieces weighing about one ounce) into a dilute solution of ethylene chlorhydrin (the exact amounts of 40 percent ethylene chlorhydrin per liter being shown in the tables), and storing the dipped pieces in covered glass jars for a definite period (usually 24 hours but sometimes only 16 or even eight hours). At the end of the storage period the treated pieces were planted in soil in flats until a subsequent time at which it was desired to remove a sample for the tests. The check lot consisted of pieces from the same lot of tubers, stored for the same length of time in the same type of containers, and handled in exactly the same manner except that they were dipped into water instead of chemical solutions. The sodium thiocyanate and thiourea treatments consisted in soaking the potatoes (cut into pieces ready for planting) for one hour in the solutions, the strength of which is described in the tables, and then planting the treated pieces in soil at once without rinsing. The chlorhydrin treatment for whole tubers consisted in placing whole tubers in glass or earthenware vessels for which covers were provided, exposing them for one day to vapors of ethylene chlorhydrin which was allowed to evaporate from pieces of cheesecloth placed loosely at the top of the vessel. The treated tubers were then stored intact (without cutting into pieces) in paper bags until samples for the tests were wanted.

### Obtaining Samples

In taking samples for analysis 25 to 50 of the cut pieces were removed from the soil, were wiped with a moist rag; then the eye of each piece was removed by first cutting away the flesh to the level of the eye and picking out the eye with a knife point, taking about one-tenth gram of tissue per eye; from the rest of the seed-piece a thin layer of the outer surface was removed. The eye-tissue was ground up in a mortar using successive quantities of added water, decanting after each grinding, until the final volume including the residue was 15 cc. for each gram of tissue taken; samples of the not-at-eye portion (called in the tables "tissue exclusive of eye-tissue") were obtained by cutting off a slice from each seed-piece until 50 to 75 grams of tissue were collected; the tissue was placed in a small cheesecloth bag, pounded in a mortar and pressed in a hand-press; the bag was again pounded and pressed; three pressings were made in all, the volume of juice obtainable being about 0.5 cc. per gram of tissue taken. The juice was not allowed to stand after pressing but samples for the various tests were taken within a few minutes.

It was found necessary to change this procedure for part of the work as follows: (1) Juice used for the study of both enzymic and non-enzymic reducing substances could not be subjected to so much aëration as this



method entailed, and for such tests the juice was obtained by passing the tissue through a food grinder, placing it in a cheesecloth bag, and pressing by hand. (2) It was found, as shown later in the tables, that the treatments, especially those with ethylene chlorhydrin, produced a juice with a pH different from that of the check lot; therefore special pressings were made in which the tissue was rolled in calcium carbonate previous to pressing.

## RESULTS

### Catalase

For the catalase determinations, usually two cc. of juice were diluted to 50 cc. with water and ten cc. of this were taken for the determination. In certain cases a different dilution ratio had to be chosen in order to give a suitable burette reading but in such cases the check juice was proportionately diluted. The apparatus and procedure described by Davis (3) were used except that the Dioxygen was neutralized by  $\text{CaCO}_3$  instead of  $\text{NaOH}$ .

The effect upon catalase obtained by treating potatoes with different concentrations of chemicals is shown in tables 1, 3, and 6, which refer respectively to ethylene chlorhydrin, sodium thiocyanate, and thiourea.

Columns 5 and 12 show that the effect of the ethylene chlorhydrin treatment was to increase greatly the catalase activity of the press-juice. Thus, favorable concentrations of chlorhydrin approximately doubled or even trebled the catalase in the cut-tuber dip-method treatments, and in the case of the whole-tuber treatments the differences between treated and check are even greater. It will be observed that with few exceptions when a graded series of chemical concentrations was used in treating the potatoes, a correspondingly graded series of catalase determinations was obtained. This is especially true of the juices obtained from the eye-tissue. It will be noted that the check readings for the whole-tuber series were lower than the checks from the cut-tuber series. Merely cutting the tuber and planting the pieces increased the catalase of the press-juice. Although, as shown in table 1, column 10, the pH of the press-juice (and even that of the juice after the dilution of 2 : 50 for the catalase determination, see column 11), was higher (less acid) for the treated than for the check, and therefore was more favorable for the catalase of the treated than of the check, the differences in catalase activity can not be explained by this difference in pH; for, when samples of both treated and check juices were brought to various pH values by the addition of acid or alkali the catalase of the treated was higher than the check at any pH value within the range of acidity encountered. Thus, in table 2 are shown the results of adjusting the juices of treated and check samples to various pH values and then determining the catalase activity. The treated juice was then found to be more active at the greatest acidity than the check juice was at the lowest acidity. Subsequent experiments showed that, even when ground with calcium carbonate in order to neutralize the acidity, the lots treated with

TABLE 1. *Effect of Different Concentrations of Ethylene Chlorhydrin Upon Dormant Potato Tubers*

Treatment	Lot No.	Conc. of Chemical per Liter	Per-cent Germ.	Juice from Tissue Exclusive of Eye-Tissue						Juice from Eye-Tissue Only			Days After Treatment Sample Taken	
				Catalase, cc. O <sub>2</sub> in 1 Min.	Peroxidase Ratio Treated Check		Methylene Blue Reduction Minutes Required	Indo-phenol cc. Absorbed	pH of Juice*		Catalase cc. O <sub>2</sub> in 1 Min.	Peroxidase Ratio Treated Check		
					Purpuro-gallin Method	Nadi-method			As Pressed	After Dilution		Purpuro-gallin Method		Nadi-method
Chlorhydrin, cut tubers, dip method, Cobbler	207	45 cc.	90	14.3	1.77	1.38	1.0	11.5	6.51	6.78	20.6	2.13	1.20	4
	208	15 cc.	70	19.1	1.86	1.04	6.0	5.0	6.34	6.78	17.0	2.27	1.70	
	209	5 cc.	27	16.7	1.57	1.11	1.0	7.0	6.34	6.68	12.8	1.49	1.11	
	210	Check	0	8.0	1.00	1.00	Neg.	2.5	6.03	6.56	5.9	1.00	1.00	
Chlorhydrin, cut tubers, dip method, Cobbler	252	45 cc.	96	21.7					See Note		19.5			4
	253	15 cc.	64	21.2							15.0			
	254	5 cc.	40	16.8							10.6			
	255	Check	8	13.3							10.0			
Chlorhydrin, whole tubers, vapor method, Cobbler	137	1.00 cc.	58	20.5	13.24	2.10	0.5	36.0	7.24	6.90	13.5			5
	138	0.20 cc.	60	9.3	4.40	1.69	1.5	14.0	6.56	6.90	14.3			
	139	0.04 cc.	13	3.2	2.11	1.24	Neg.	4.0	6.17	6.73	9.2			
	140	0.008 cc.	0	2.8	1.20	0.94	Neg.	2.0	6.05	6.64	5.8			
	141	Check	0	1.6	1.00	1.00	Neg.	2.5	6.00	6.56	3.9			7
Chlorhydrin, whole tubers, vapor method, Bliss	240	0.50 cc.	66	18.9	2.82	Lost					21.7	3.37		
	241	0.16 cc.	20	9.1	2.50	2.11					10.0	2.36		
	242	0.06 cc.	0	4.3	1.46	1.55					7.5	2.14		
	243	Check	12	2.9	1.00	1.00					3.9	1.00		
Chlorhydrin, whole tubers, vapor method, Bliss	193	1.00 cc.	59		2.15		0.5							7
	194	0.25 cc.	18		1.38		8.0							
	195	0.06 cc.	27		1.09		30.0							
	196	Check	0		1.00		Neg.							

Note: Tissue for lots 252-255 ground with calcium carbonate.  
 \* pH measurements made with quinhydrone electrode.

ethylene chlorhydrin had a greater catalase activity than the corresponding checks.

TABLE 2. *Effect of pH Upon the Catalase Activity of Potato Juice*

				Catalase, cc. O <sub>2</sub> in 1 min.	
				Juice from Treated Potatoes	Juice from Check Potatoes
Juice adjusted to pH	5.96	.....		12.8	1.4
"	6.10	.....		16.4	2.9
"	6.50	.....		18.3	5.4
"	6.95	.....		19.7	7.7

The pH of the juice during the process of pressing and making the catalase measurement is not capable of explaining the increased activity of the juice from the treated lots; but the higher pH value of the sap which may have prevailed in the potato between the time of treatment and time of sampling may be of great importance in protecting the catalase formed or in inducing a greater production of catalase in the treated lots. These experiments throw no light on this point.

The increases in catalase in potatoes treated with ethylene chlorhydrin were not due to the direct effect of the ethylene chlorhydrin upon the catalase already present. This was shown by experiments in which various amounts of ethylene chlorhydrin were added to potato juice and the catalase activity subsequently measured. In no case were any increases observed; decreases were found if the amount of chemical added was large enough. Thus eight cc. of 40 percent ethylene chlorhydrin per 100 cc. of potato juice caused a decrease in the catalase, but when the amount added was less than about two cc. per 100 cc. of juice no effect upon the catalase was noted.

The effect of sodium thiocyanate treatment of potatoes upon the catalase of press-juice is shown in table 3, columns 5 and 12. In the determinations with juice from tissue exclusive of eye-tissue (column 5) it is seen that small and probably insignificant differences were found between the check lots and the lots treated with various concentrations of chemical. Only when we examine the data on juices from eye-tissue, column 12, do we find any suggestion that the catalase activity has been increased, and, even here, the differences are small, and hardly beyond the experimental error.

Preliminary experiments had shown that the addition of small amounts of sodium thiocyanate to potato juice greatly depressed the catalase activity, even ten milligrams of NaSCN per 100 cc. of potato juice causing a marked reduction. It was thought possible that, in the process of soaking the potatoes in sodium thiocyanate solutions, enough chemical could be present in the press-juice to have a retarding influence upon catalase. Tests showed that thiocyanate was present in appreciable amounts in the juice from the treated potatoes. Consequently, the juices were dialyzed in collodion bags in running water, under which conditions the catalase is retained within the bag and the thiocyanate together with other easily diffusible substances passes through. This permitted a separation of the

TABLE 3. *Effect of Different Concentrations of Sodium Thiocyanate Upon Dormant Potato Tubers*

Treatment	Lot No.	Conc. of Chemical Percent	Per- cent Germ.	Juice from Tissue Exclusive of Eye-Tissue						Juice from Eye-Tissue Only				Days After Treat- ment Sample Taken
				Catalase, cc. O <sub>2</sub> in 1 Min.	Peroxi- dase Ratio Treated Check		Methylene Blue Reduction Minutes Required	Indo- phenol cc. Ab- sorbed	pH of Juice*		Catalase, cc. O <sub>2</sub> in 1 Min.	Peroxi- dase Ratio Treated Check		
					Purpu- ro- gallin Method	Nadi- method			As Pressed	After Dilution		Purpu- ro- gallin Method	Nadi- method	
Cut tubers, soaked 1 hour, Cobbler	236	1.00	100	9.0	2.15	1.59	30.0	11.0	See Note		9.3	2.38	1.59	5
	237	0.50	80	8.2	1.77	1.20	Pos.	8.0			6.9	1.79	1.20	
	238	0.25	65	8.7	1.21	1.20	Sl.	2.5			7.3	2.02	1.20	
	239	Water	0	8.5	1.00	1.00	Neg.	2.5			6.5	1.00	1.00	
Cut tubers, soaked 1 hour, Bliss	182	1.00	100	7.3	2.31	1.20	Pos.	10.0	6.10	6.64	9.0	1.50	1.19	4
	184	0.50	84	7.2	1.40	1.32	Sl.	8.0	5.97	6.68	11.2	0.89	0.92	
	186	0.25	68	6.0	1.40	0.92	Pos.	2.5	5.88	6.75	7.1	1.19	0.92	
	183	Water	12	6.4	1.00		Neg.		5.93	6.64	8.5	1.00		
	185	Water	16	8.2	1.36	1.00	Neg.	2.5	5.88	6.68	7.5	0.99	1.00	
Cut tubers, soaked 1 hour, Bliss	167	1.00	100	11.2	1.50	1.57	15.0	6.5	5.97	6.51	6.3	1.34	1.32	5
	168	0.50	84	10.5	1.36	1.64	Pos.	4.0	5.88	6.44	5.8	1.03	1.08	
	169	0.25	60	6.8	1.04	1.22	Sl.	2.5	5.83	6.44	3.2	0.87	1.02	
	170	0.13	40	7.6	0.90	1.10	Neg.	1.0	5.68	6.22	2.6	0.77	0.81	
	171	Water	8	7.1	1.00	1.00	Neg.	1.5	5.97	6.31	4.9	1.00	1.00	
Cut tubers, soaked 1 hour, Cobbler	157	2.00		3.5	1.48	1.44	Pos.		6.00	6.47	3.9		1.16	4
	158	0.67		5.5	1.26	1.19	Sl.		5.93	6.31	5.5		1.12	
	159	0.22		6.3	1.24	1.15	Neg.		6.00	6.31	5.2		1.04	
	160	Water		5.1	1.00	1.00	Neg.		5.97	6.31	3.2		1.00	

Note: Tissue for lots 236-239 ground with calcium carbonate for the catalase determination.  
 \* pH measurements made with quinhydrone electrode.

catalase and the thiocyanate which, if present, could retard it. The results are shown in table 4 from which it can be seen that, although before dialysis there was no graded series of catalase readings corresponding to the series of concentrations of sodium thiocyanate, after dialysis the catalase activities

TABLE 4. *Effect of Dialysis of Potato Juice From Tubers Treated With Chemicals*

Treatment Applied	Lot No.	Conc. of Chemical Used	Catalase, cc. O <sub>2</sub> in 1 Min.	
			Before Dialysis	After Dialysis
NaSCN, cut tubers, soaked one hour, Cobbler	236	1.00%	9.0	15.9
	237	0.50%	8.2	12.0
	238	0.20%	8.7	8.4
	239	Water	8.5	5.8
Thiourea, cut tubers, soaked one hour, Bliss	224	1.00%	9.1	8.8
	225	0.50%	9.7	8.8
	226	0.25%	9.8	8.2
	227	Water	9.5	8.4
Chlorhydrin, whole tubers, vapor method, Bliss	240	0.50 cc.	18.9	19.8
	241	0.16 cc.	9.1	10.0
	242	0.06 cc.	4.3	3.8
	243	Check	2.9	2.9

then arranged themselves in a series in an order agreeing with the strength of the chemicals.

Further tests were made to determine the effect of length of dialysis upon the catalase activity of juices from the sodium thiocyanate treatments. These results are given in table 5 which shows that a period of dialysis as short as one-half hour greatly increased the catalase of the juice from thiocyanate-treated tubers, and that the maximum effect was reached in about two hours. Dialysis for 16 hours reduced the catalase of both treated and check, but the treated remained higher than the check.

TABLE 5. *Time Relations in Dialysis of Juice From Potatoes Treated With Sodium Thiocyanate*

Time After Beginning of Dialysis	Catalase, cc. O <sub>2</sub> in 1 Min.			
	Lot I		Lot II	
	Treated	Check	Treated	Check
Start.....	6.0	13.6	7.0	7.4
½ hour.....	15.7	14.3		
2 hours.....	19.9	14.9	18.6	11.8
4 hours.....	20.0	11.6	14.7	10.0
16 hours.....	11.0	4.7	9.7	5.2

These results indicate that the effect of the sodium thiocyanate solution upon the catalase of potato was two-fold: (1) It induced the formation of

TABLE 6. *Effect of Different Concentrations of Thiourea Upon Dormant Potatoes*

Treatment	Lot No.	Conc. of Chemical Percent	Per-cent Germ.	Juice from Tissue Exclusive of Eye-Tissue						Juice from Eye-Tissue Only			Days After Treat-ment Sample Taken	
				Catalase, cc. O <sub>2</sub> in 1 Min.	Peroxi-dase Ratio Treated Check		Methylene Blue Reduction Minutes Required	Indo-picol Ab-sorbed	pH of Juice*		Catalase cc. O <sub>2</sub> in 1 Min.	Peroxi-dase Ratio Treated Check		
					Purpuro-gallin Method	Nadi-method			As Pressed	After Dilution		Purpuro-gallin Method		Nadi-method
Cut tubers, soaked 1 hour, Cobbler	228	1.00	96	12.7	1.50	1.41	Neg.	3.0	See Note		22.6	2.47		5
	229	0.50	72	12.4	1.44	1.38	Neg.	5.5			26.0	1.47		
	230	0.25	8	13.2	1.37	1.20	Pos.	7.0			21.6	1.64		
	231	Water	12	10.8	1.00	1.00	Sl.	2.5			15.9	1.00		
Cut tubers, soaked 1 hour, Bliss	224	1.00	92	9.1	1.21	1.50	Sl.	18.0	See Note		25.6			5
	225	0.50	24	9.7	1.14	1.57	Sl.	11.0			16.4			
	226	0.20	12	9.8	1.21	1.45	Pos.	11.0			16.0			
	227	Water	16	9.5	1.00	1.00	Neg.	8.5			13.8			
Cut tubers, soaked 1 hour, Bliss	177	1.00	88	3.6	2.04	1.33	15.0	5.0	6.05	6.98	8.0			3
	178	0.40	76	3.1	1.20	1.50	Neg.	3.0	5.97	6.64	3.6			
	179	0.16	36	1.4	1.23	1.39	Neg.	2.5	5.97	6.51	3.3			
	180	0.06	0	0.7	0.69	1.08	Neg.	2.5	5.93	6.47	3.4			
	181	Water	0	2.0	1.00	1.00	Neg.	2.5	5.88	6.47	4.6			
Cut tubers, soaked 1 hour, Bliss	162	2.00	100	8.9	2.04	1.42	2.0	9.5	6.14		16.5	2.27	1.18	4
	163	1.00	95	10.2	1.72	1.22	1.5	4.0	6.10		17.9	1.82	1.14	
	166	0.50	100	9.2	1.39	1.37	Neg.	3.5	6.05		12.4	1.44	1.08	
	165	0.25	45	10.2	1.53	1.20	Neg.	3.5	6.00		9.7	1.81	1.00	
	164	Water	20	7.3	1.00	1.00	Neg.	2.5	5.97		8.7	1.00	1.00	
Cut tubers, soaked 1 hour, Cobbler	152	2.00		7.8	1.52	1.38	Pos.				23.6	2.57		4
	153	0.67		8.8	1.76	1.35	Neg.				15.7	1.44		
	154	0.22		8.0	1.55	1.02	Neg.				10.2	1.07		
	155	Water		7.8	1.00	1.00	Neg.				7.8	1.00		

Note: Tissue for lots 224-231 ground with calcium carbonate for the catalase determination.

\* pH measurements made with quinhydrone electrode.

an increased amount of catalase in the press-juice. (2) But the thiocyanate absorbed by the tissue, when pressed out with the juice, interfered with the action of the catalase that had been formed. Whether the thiocyanate was interfering with the catalase within the potato tissue before the juice was pressed out is an important question upon which the present experiments furnish no evidence.

The effect of thiourea treatments upon catalase is shown in table 6. The data from the juice obtained from the seed-pieces after excluding the eye-tissue are shown in column 5 and indicate no differences in catalase activity between treated and checks, and no series of readings corresponding to the concentrations of chemicals used. Furthermore when these juices were subjected to dialysis as was done for the thiocyanate lots (see table 4) no differences between treated and checks were obtained.

The catalase determinations on the extracts from the eye-tissue (column 12), however, show definite increases of treated over checks and a fair agreement between the gradation of catalase values and the series of concentrations of thiourea; but, as compared with either ethylene chlorhydrin or sodium thiocyanate, thiourea had much less effect upon the catalase of the juices.

The direct action of thiourea upon catalase of potato juice is not important in connection with these tests. The addition of thiourea to potato juice in amounts greater than about 150 milligrams per 100 cc. of juice retarded catalase activity; amounts smaller than about 50 milligrams produced no effect. Loevenhart and Kastle (8) found a marked increase in the catalase of hog's liver by the addition of thiourea, but a similar effect upon potato catalase was not observed by us.

### Peroxidase

Two different methods were used in determining the peroxidase activity: the purpurogallin method and a modification of the Nadi-oxidase reaction. In the purpurogallin method ten cc. of five per cent pyrogallol, 30 cc. of phosphate buffer at pH 6.5, two cc. of 12 volume  $H_2O_2$ , and the required amount of potato juice or extract were added in centrifuge tubes. The mixture was allowed to stand in a constant temperature room either at 23° C. or at 0° C. according to whether it was more convenient to stop the reaction after a few hours or allow it to continue overnight. When the precipitate of purpurogallin, which formed as a result of the peroxidase action of the juice, became large enough to give a good colorimeter reading, the tubes were centrifuged and the precipitate was dissolved in 95 percent alcohol with the aid of heat, was made up to volume, was filtered, and the amount of purpurogallin was estimated by a colorimetric comparison using the check lot as the standard. Usually either one or two cc. of potato juice and five to ten cc. of extract from the eye-tissue gave suitable concentrations for convenient measurements, and, although the amount used

varied somewhat in different tests, in any one test the amounts of juice used and all other conditions of the procedure were exactly the same for the treated and check lots.

In the Nadi method 25 cc. portions of the paraphenylene diamine-alphanaphthol mixture, made up in a citrate buffer of pH 4.5, were placed in centrifuge tubes with 25 cc. of toluene. Sufficient extract or juice to give a convenient reading (usually 1 cc.) and 5 cc. of Dioxygen diluted 1 : 20 were added. The tubes were stoppered and allowed to stand with frequent shaking until sufficient color developed for a satisfactory colorimetric comparison. The peroxidase of the juice brings about the production of indophenol by interaction of the reagents; the indophenol dissolves in the toluene giving a red color which varies in intensity according to the peroxidase activity of the juice tested. The tubes are centrifuged. The top layers are decanted and compared in a colorimeter, using the check as the standard.

The agreement between the two methods was only fair, and, in some cases, quite poor; this divergence is not surprising, since the enzyme not only acted upon different substrates in the two cases, but also at two different hydrogen-ion concentrations. But, in general, the principal conclusions that could be reached by a consideration of the data were the same by either method.

The effect of the chemical treatments upon the peroxidase of the press-juice is shown for the ethylene chlorhydrin treatments in table 1, for sodium thiocyanate in table 3, and for thiourea in table 6. In the case of the peroxidase it appears unnecessary to discuss the three chemicals separately as was done for catalase, since the chemicals gave more nearly equal results. The data show in general an increase of the treated over the check, the most favorable concentrations of chemicals giving an increase of about 50 to 100 percent. The relationship between the concentration of the chemical and the peroxidase ratio, however, is not such as to give gradations which correspond exactly to the concentrations of chemical used in the treatment; the agreement is fair but there are several irregularities.

None of the three chemicals had an accelerating effect upon the peroxidase when added directly to potato juice. Ethylene chlorhydrin could be added up to about one percent of the potato juice (by volume) without either increasing or decreasing the peroxidase activity. Sodium thiocyanate at about 50 milligrams per 100 cc. of juice retarded, as did also thiourea at about 200 milligrams, but in the case of neither chemical did lower concentrations cause a detectable increase in peroxidase as measured by the purpurogallin method.

### **Time Relations in the Development of Catalase and Peroxidase Activity**

In order to determine approximately how soon after treatment the increases in catalase and peroxidase began, samples of treated and check



potatoes were taken at intervals of 16, 24, 48, etc. hours after treatment. The results for the ethylene chlorhydrin treatments are shown in table 7 and for sodium thiocyanate and thiourea in table 8. In the chlorhydrin treatments it is seen that the catalase increase is very marked between the 24th and 48th hour and increases as early as the 16th hour after treatment were observed. The increases were greater in the extracts from the eye-tissue than in juices from the not-at-eye portion.

TABLE 7. *Time Relations in Effect of Ethylene Chlorhydrin Treatment of Dormant Potatoes*

Lot No	Variety	Hours After End of Treatment	Juice from Tissue Exclusive of Eye-Tissue			Juice from Eye-Tissue Only		
			Catalase, cc. O <sub>2</sub> in 1 Min.		Peroxidase Ratio. Check = 1.00	Catalase, cc. O <sub>2</sub> in 1 Min.		Peroxidase Ratio. Check = 1.00
			Treatment	Check		Treatment	Check	
187 and 188	Bliss	24	10.1	16.0	1.07	4.9	8.2	1.82
		48	16.9	11.0	2.05	24.9	5.5	2.17
		72	24.5	12.2	2.04	24.3	6.4	3.45
		96	22.2	11.3	2.20	24.6	7.0	6.12
191 and 192	Cobbler	24	9.1	7.9	1.28	10.7	9.3	1.63
		48	15.9	9.0	1.74	29.8	11.3	2.50
		72	21.2	8.6	2.68	28.7	12.2	2.52
211 and 212	Cobbler	30	10.2	5.5		6.5	3.5	
		56	15.1	7.5		21.5	5.3	
		80	13.1	4.3		25.1	6.1	
213 and 214	Bliss	30	6.5	5.7		8.6	2.2	
		56	14.3	7.5		20.5	3.6	
		80	14.8	5.5		29.8	5.7	
215 and 216	Cobbler	16	4.4	1.7	2.20	4.9	3.0	1.77
		23	7.6	3.8	1.98	4.0	3.3	1.11
		42	15.7	6.8	2.38	15.0	4.6	1.45
219 and 220	Cobbler	0	3.7	3.7	1.01	4.5	4.8	1.23
		16	9.3	5.8	1.96	5.6	2.3	1.23
		24	9.0	4.9	1.64	6.7	3.6	1.32
		40	18.7	4.1	2.86	25.4	5.6	2.02

The peroxidase increases developed in a manner similar to that of the catalase, and although the course of development of peroxidase was not as consistent in the different lots as was the catalase, there was some evidence that the peroxidase increase began even earlier than that of the catalase.

The results with thiourea (table 8) show no consistent increase with respect to time in catalase in the juice from the tissue-not-at-eye, but in the extract from the eye-tissue there is a gradual increase beginning about the 24th to 48th hour; this is true also of the peroxidase, except that the increase in the eye-tissue samples is not gradual but rather uneven. The sodium thiocyanate data on this point (table 8) are in agreement with

TABLE 8. *Time Relations in Effect of Sodium Thiocyanate and Thiourea Treatment of Dormant Potatoes*

Lot No.	Treatment	Hours After End of Treatment	Juice from Tissue Exclusive of Eye-Tissue				Juice from Eye-Tissue Only			
			Catalase, cc. O <sub>2</sub> in 1 Min.			Peroxidase Ratio, Check = 1.00	Catalase, cc. O <sub>2</sub> in 1 Min.			Peroxidase Ratio, Check = 1.00
			Thiourea Treatment	NaSCN Treatment	Check	Thiourea Treatment	Thiourea Treatment	NaSCN Treatment	Check	Thiourea Treatment
221 to 223	Cut tubers, soaked 1 hour, Cobbler	0	3.6	4.2	4.8	0.93	4.2	3.6	3.9	0.99
		24	6.3	4.5	6.2	1.01	6.9	4.6	4.8	1.12
		48	8.7	3.7	8.2	1.21	8.6	8.2	7.3	1.52
		72	7.7	5.4	7.4	0.95	8.6	8.3	6.8	1.36
		96	7.6	4.9	6.7	1.21	8.2	6.2	5.8	1.33
		120	8.5	6.3	7.2	0.95	12.8	8.7	7.1	1.38
189 and 190	Cut tubers, soaked 1 hour, Bliss	0								
		24		6.3	11.9			5.6	9.1	1.04
		48		4.9	8.8			6.1	11.4	1.28
		72		4.7	7.7			3.6	8.1	1.99
		96		5.5	7.8			11.2	11.2	1.90
		120		10.7	11.6			15.0	7.2	2.03
		148								3.34

those in table 3 in showing no catalase increases in the juice from the not-at-eye tissue at any time during the period of sampling; but, as described in a previous paragraph, this is no doubt due to the inhibiting effect on the catalase resulting from the presence of thiocyanate in the press-juice. It will be remembered that after dialysis of such juices to remove the NaSCN the treated juices were then higher than the corresponding checks. In the eye-tissue samples also, there was no increase in catalase, at least not until after 120 hours. The peroxidase measurements with the exception of one lot show increases beginning about the 72nd or 96th hour.

### Reducing Properties of Juices

#### *Methylene Blue Reduction*

The solution of methylene blue contained 50 milligrams per liter. To five cc. of the freshly-pressed juice in a narrow test tube one to two cc. of the methylene blue solution were added and the two were mixed. The time required for complete decoloration of the liquid at the bottom of the tube was noted. In cases where the reduction was not complete but only partial, the designations *Sl.* (= slight) and *Pos.* (= positive) were used to distinguish them from the lots called *Neg.* (= negative) in which no reduction at all could be noted at the end of 30 minutes.

The effect of treatment with different concentrations of ethylene chlorhydrin upon the methylene blue reducing capacity of potato juice is shown in table 1, column 8. Under the conditions of the test, juices from the check lots were not able to reduce methylene blue within 30 minutes. The lots treated with favorable concentrations of ethylene chlorhydrin, however, caused reduction within about a minute or less.

Treatments with sodium thiocyanate and thiourea gave juices with much less capacity to reduce methylene blue, as shown in table 3, column 8 and table 6, column 8. In these cases the reduction was greater with the treated than with the checks, but the differences were not as great as with the chlorhydrin treatments.

The pH value of the solution in which the methylene blue reduction is carried out is of importance, the less acid the solution the more rapid the rate of reduction. This was taken into account and it was found that when the treated and check juices were adjusted to the same pH the reduction was always more rapid in the treated lot; furthermore, while check juices were unable to cause reduction at pH values more acid than about 6.20, chlorhydrin-treated juices made acid to 5.75 could still reduce the methylene blue within the 30 minute period. The addition of small quantities of ethylene chlorhydrin, sodium thiocyanate, or thiourea to potato juice did not increase the rate of reduction of methylene blue. Sodium thiocyanate at about 20 milligrams per 100 cc. of juice appeared to retard the action somewhat.

### *Indophenol Reduction*

The indophenol solution was prepared by dissolving 100 milligrams of indophenol in 25 cc. of boiling alcohol and diluting rapidly with water to one liter. When five cc. of potato juice are placed in a test tube and the indophenol solution is run in from a burette, the blue color is at first instantly reduced to a colorless condition; upon the continuous addition of the dye a point is reached at which the blue color is not discharged. The amounts of indophenol decolorized by juices from the potatoes treated with different concentrations of chemicals are shown in tables 1, 3, and 6. It is seen that, while juices from untreated lots absorb about 2.5 cc. of indophenol under the conditions of the test, the juices from the lots treated with favorable concentrations of chemicals absorb 5 to 36 cc. In all tests but one there was a good correlation between the concentration of chemical used in treating the potato and the indophenol absorption of the press-juice. The order of effectiveness of the three chemicals is first, ethylene chlorhydrin, then sodium thiocyanate, and finally thiourea; but even in the case of thiourea the value for the treated is about twice that of the check. The indophenol absorption of juices obtained from the whole tuber treatments with ethylene chlorhydrin was especially high.

It should be emphasized that this reaction can be obtained when boiled juice is used, and, therefore, differs to a certain extent from the methylene blue reduction which is enzymatic, and does not occur in boiled juice.

### *Self-reducing Properties*

When potato juice is allowed to come in contact with air it becomes first reddish, and later brown, or even nearly black. In these experiments, during the process of extraction the juices were exposed to the air and became more or less dark brown. When these juices were poured into test tubes, they gradually became lighter in color, and finally nearly canary yellow. One of the most distinct results of the chemical treatments was to favor the rapidity of this self-reduction; and, furthermore, the order in which the juices became decolorized was in the order of the series of concentrations of chemicals used in treating the potatoes. With all three chemicals it was observed that the juice from the highest concentration of chemical became yellow in the shortest time, and then followed in order the different treatments, the check lot remaining brown for the longest time. The naturally-occurring pigment-forming system, therefore, was a better indicator than methylene blue for distinguishing between different concentrations of chemicals in their effect upon the reducing properties of juices. Also, the three chemicals were more nearly alike with respect to this self-reduction than they were toward methylene blue reduction. Thus, in the thiourea treatments the differences between treated and check were not large, as shown in table 6; but the capacity of thiourea treatments to give quicker reduction of the color of oxidized juices was very distinct, and

gave gradations with respect to these characteristics corresponding closely to the graded series of concentrations of chemicals.

It is likely that the capacity of dark colored juices from thiourea-treated potatoes to decolorize when allowed to stand is connected with the ability of thiourea to retard darkening of juices or tissue when the thiourea is added directly to the juice, or when pieces of potato tissue are dipped into solutions of thiourea and allowed to stand in air. In such cases the darkening can be completely inhibited if the proper concentration is chosen.

### *Iodine Reduction*

When ten cc. of ten percent trichloroacetic acid are added to five cc. of potato juice the mixture will absorb appreciable amounts of 0.01 *N* iodine. Using starch as an indicator of an excess of iodine, it was found

TABLE 9. *Reducing Properties of Juice From Potatoes Treated With Chemicals*

Chemical Treatment	Lot No	Conc of Chemical Used	0 or <i>N</i> Iodine Absorbed cc.	Reduction of Phosphotungstic Reagent. Ratio Treated Check
NaSCN, cut tubers, soaked 1 hour	167	1.00%	1.55	2.02
	168	0.50%	1.25	1.54
	169	0.25%	1.00	1.48
	170	0.13%	0.75	0.87
	171	Water	0.70	1.00
NaSCN, cut tubers, soaked 1 hour	236	1.00%	1.65	1.95
	237	0.50%	1.50	1.78
	238	0.25%	1.05	1.38
	239	Water	0.85	1.00
Chlorhydrin, whole tubers, vapor method	137	1.00 cc.	2.75	
	138	0.20 cc.	1.15	
	139	0.04 cc.	0.30	
	140	0.008 cc.	0.20	
	141	Check	0.20	
Chlorhydrin, whole tubers, vapor method	193	1.00 cc.	2.10	1.79
	194	0.25 cc.	1.00	0.98
	195	0.06 cc.	1.15	1.00
	196	Check	0.85	1.00
Chlorhydrin, cut tubers, dip method	207	45 cc.	0.75	
	208	15 cc.	0.85	
	209	5 cc.	0.75	
	210	Water	0.45	
Chlorhydrin, cut tubers, dip method	187	30 cc.	2.00*	
	and	Check	1.05	
	188	30 cc.	3.30	
		Check	0.90	
Thiourea, cut tubers, soaked 1 hour	162	2.00%		2.36
	163	1.00%		1.13
	165	0.50%		Lost
	166	0.25%		1.13
	164	Water		1.00

\* First pair of readings 48 hours, and second pair 72 hours after treatment.

that juices from the potatoes that had been treated with ethylene chlorhydrin and sodium thiocyanate absorbed larger quantities of iodine than juice from untreated potatoes. The results are shown in table 9. The thiocyanate-treated lots absorbed about twice as much iodine as the checks, and for the ethylene chlorhydrin treatments the divergence was even greater. The amounts of iodine absorbed by the juices formed in the different tests a series of values which corresponded fairly well with the amounts of chemicals used in treating the potatoes. Sodium thiocyanate itself will absorb iodine in neutral or alkaline solutions, but not in the acidity produced by the amounts of trichloroacetic acid added in making the tests.

It will be noted that no results with thiourea are shown. This is because thiourea itself absorbs iodine in acid solution, hence although this test was made, and, although the juices from the treated lots absorbed more iodine than the checks, it seemed that the procedure was more nearly a measure of the amount of thiourea absorbed by the potato than of the substances for which the iodine test was being used.

Titration with 0.01 *N* iodine in acid solution, using nitroprusside as an indicator, has been recommended as a measure of glutathione (see Tunnicliffe, 12). Starch has also been tried as an indicator, but generally regarded as unsatisfactory, merely because it gives higher results than nitroprusside. It has, however, been recommended by Blanchetiere and Melon (1). Potato juice gives a very feeble nitroprusside reaction and therefore nitroprusside can not be used as an indicator. It is probable that the substance responsible for the iodine reaction in the potato is not glutathione, but resembles the reducing substance described by Szent-Györgyi (10).

#### *Phosphotungstic Reagent Reduction*

If to two cc. of Folin's (6) improved uric acid reagent (free from the phenol reagent) are added two cc. of boiled filtered potato juice, together with ten cc. of 20 percent sodium carbonate, a blue color will develop within a few minutes because of the reduction of the tungstic reagent. It was found that the intensity of this blue color was greater with juices from the potatoes that had been treated with chemicals than with the check lots, and that a colorimetric comparison of treated and check could be made. The results are shown in table 9 from which it is seen that the reducing action of the juice from potatoes receiving favorable amounts of chemical was approximately twice that of the check juices.

#### *Effect of Method of Extraction, Time of Standing, and Aëration Upon Reducing Properties*

The reducing capacity of a juice decreased to low values when the juice was exposed to air in a thin layer. Under these conditions its capacity to reduce methylene blue was completely lost, and its effectiveness in

TABLE 10. *Relation of Reducing Properties to the Method of Extraction and Time of Standing of Juice*

Lot No.	Treatment	Cubic Centimeters of 0.01 N Iodine Absorbed						Indophenol Reduced cc.		Reduction* of Phosphotungstic Reagent		Reduction of Methylene Blue, min.
		Amount of Chemical	Press-juice			Trichloro-acetic Acid Extract of Tissue	Boiling Water Extract of Tissue	Fresh Juice	Boiling Water Extract of Tissue	Juice	Trichloro-acetic Acid Extract of Tissue	
			At Once	Stood ½ hr.	Stood 1 hr.							
197	Ethylene chlorhydrin, whole tubers, vapor method	1 cc.	2.50	3.10	3.35	3.10	2.65	18	22	2.00	1.16	Fresh Juice 1/4 min.
198	Ethylene chlorhydrin, whole tubers, vapor method	1/4 cc.	2.10	2.45	2.55	3.00	2.70	13	22	1.51	1.18	3 min.
199	Ethylene chlorhydrin, whole tubers, vapor method	1/16 cc.	1.10	1.15	1.15	2.40	2.25	6	21	0.70	0.93	Not complete after 30 min.
200	Not treated	Check	1.50	1.80	1.55	2.65	2.20	4	21	1.00	1.00	Not complete after 30 min.

\* Colorimetric comparison with check at 1.00 as standard.

reducing iodine and indophenol was much lower. In addition, the reducing property of a juice was found to depend upon whether the tests were applied upon press-juice or upon extracts obtained with boiling water or with trichloroacetic acid. The results of a series of tests on the behavior of the reducing capacity are shown in table 10.

The following observations are to be considered: (1) In treated juices the power to reduce 0.01 *N* iodine increased on standing. (2) Treated juices reduced methylene blue and this action was inhibited by boiling. (3) The reducing action of the juice on indophenol and 0.01 *N* iodine disappeared rapidly on aerating the fresh juice, but not the boiled juice. (4) Extracts prepared by dropping the tissue into boiling water, or extracting with trichloroacetic acid, show much smaller differences when titrated with 0.01 *N* iodine than the expressed juice. These results would be in agreement with the assumption that the substance or substances responsible for the indophenol, iodine, and phosphotungstic reduction are present in the tissue in the reduced form; that these are partially oxidized in the process of extraction, but reduced back again in the treated juice.

### Amylase and Invertase

The activity of both these enzymes may be measured by their effect in causing an increase in reducing sugar by the splitting of added substrates. But the measurements are difficult with potato juice for the reason that the amylase activity is not high and the correction due to the sugar in the blank test is relatively high in comparison with the increase it is desired to measure. Invertase may be studied simultaneously but, to make a comparison between treated and check juices in our experiments, it was necessary to add cane sugar in excess to the juice, since juices from lots treated with chemicals contained initially higher amounts of cane sugar than the check lots.

To estimate the amylase and invertase activity of a given juice at least four sugar determinations (eight or twelve including duplicates) were needed for each lot of juice. Since there were usually four or more lots in each experiment, the time required for performing the necessary operations made it impossible to carry out more than preliminary determinations of amylase and invertase measurements. The results of such tests are shown in tables 11 and 12. It is seen (columns 7 and 8 in table 11) that the amylase and invertase activities of the treated lots were higher than the check, and that a series of graded values was obtained corresponding well with the gradation of chemical concentrations used in treating the potatoes. Column 6 shows that the sucrose values were higher in the treated than in the check. But from column 3 it will be seen that the pH values were also different in the treated and check lots, and it was necessary to take this into account in another experiment. In order to determine the effect of the pH value of the juice upon amylase and invertase activity, a portion of the juice from the treated lot was adjusted to the pH of the



check lot by the addition of acid, and the pH of a portion of the check lot was adjusted to that of the treated lot by the addition of alkali. The amylase and invertase activity of the four lots were then determined and the results are shown in table 12. It is clear that the difference in pH can not account for the difference in enzym action between treated and

TABLE 11. *Effect of Ethylene Chlorhydrin Treatment Upon Amylase and Invertase of Potato*

Treatment	Conc. of Chem. cc. per l	pH	Potassium Permanganate Values* Resulting from Sugar Determination. cc N/20 KMnO <sub>4</sub> per 5 cc. of Juice				
			Boiled Juice	Juice Increase on Standing	Sucrose† Present	Amylase Values‡	Invertase Values§
Chlorhydrin, whole tubers, vapor method	1.00	6.73	0.00	2.30	22.15	22.25	11.23
	0.33	6.47	0.17	4.16	21.38	21.44	9.09
	0.11	6.47	Trace	3.78	18.40	20.40	7.90
	0.09	6.51	0.93	3.39	15.32	19.36	6.16
	Check	6.34	2.92	1.18	13.53	16.63	6.10

\* Averages of three duplicate determinations.

† Representing the relative amounts of sucrose in the juices.

‡ Representing the increase in reducing power due to hydrolysis of added starch after making correction for blanks.

§ Represent the increase in reducing power due to inversion of added sucrose after making correction for blanks.

TABLE 12. *Effect of pH on the Comparison of Treated and Check Lots With Respect to Amylase and Invertase*

Treatment		Potassium Permanganate Values Resulting from Sugar Determinations cc. N/20 KMnO <sub>4</sub> per 5 cc. Juice				
		Boiled Juice	Juice Increase on Standing	Sucrose Present	Amylase Values	Invertase Values
Chlorhydrin, whole tuber, treatment 1 cc. per l. 24 hrs.	Juice as pressed pH = 6.8	0.15	1.80	22.45	20.33	7.13
	Juice adjusted to pH of check lot = 6.3	0.16	1.30	24.34	16.20	5.07
Check, not treated	Juice as pressed pH = 6.3	6.40	1.61	15.2	7.07	1.90
	Juice adjusted to pH of treated lot = 6.8	6.33	1.77	14.3	15.55	3.35

Note: See foot-notes to table 11 which apply here also.

check since even at the same pH the treated was 50 to 100 percent higher than the check.

It is realized that further work is needed before conclusions can be drawn, and in particular these experiments, which were carried out with

ethylene chlorhydrin, should be extended to other chemicals. The effect upon amylase of the chemicals themselves when added to potato juice is now being investigated, especially with regard to sodium thiocyanate, because of the previous work of Johnson and Wormall (7) which indicated a direct stimulative effect of this chemical upon the amylase of the saliva and potato juice, at any rate upon the dialyzed enzym.

### Acetaldehyde Formation

Boresch (2), who investigated the effect of the warm-bath treatment of twigs of woody plants in breaking dormancy, found increased amounts of acetaldehyde in treated tissue, and he believed that the aldehyde formed was an effective agent in inducing growth of buds. We made some preliminary measurements of acetaldehyde in treated and check lots of potatoes, first steam distilling the tissue to separate the acetaldehyde, and then using the method of Tomoda (11). The test can not be regarded as specific for acetaldehyde since it is given by any aldehyde or ketone. The aldehyde measurement by this method can be expressed in terms of cubic centimeters of 0.1 *N* iodine. Only small amounts of acetaldehyde were found, but in the three tests made the ethylene chlorhydrin treated potatoes gave higher values than the check lots. Thus, using 250 grams of minced tissue for each test, the titration values were: treated 0.85 cc., check 0.25 cc.; treated 0.45 cc., check 0.30 cc.; treated 0.60 cc., check 0.30 cc. The effect of sodium thiocyanate and thiourea treatments upon the development of acetaldehyde in potatoes was not determined.

Because of the low values obtainable from even such a large amount of tissue, it was concluded that neither time nor tissue could be sacrificed for further tests of acetaldehyde at that time.

But even if it could be demonstrated that larger amounts of acetaldehyde are formed in treated tissue, it could not be concluded from this that acetaldehyde was the cause of the breaking of dormancy. Since acetaldehyde is an intermediate product in respiration, and since Smith (9) has shown that the ethylene chlorhydrin treatment greatly increases the respiration of potato tubers, a more likely explanation is that the acetaldehyde is a result of the increased life activity, and not a cause of its initiation. In other words the dormancy has been broken and considerable cell activity has occurred before the increase in acetaldehyde starts to take place.

### Effect of Presence and Absence of Eyes in the Chemical Treatment of Potato Tubers

To determine whether the changes that occur when the cut tubers are treated with chemicals also take place in the absence of eyes, the eyes were removed from a portion of the seed-piece by reaming out with a knife blade. The two lots (with and without eyes) were then subjected to the same chemical treatment, and after several days were compared with each

TABLE 13. *Effect of Presence and Absence of Eyes in the Chemical Treatment of Dormant Potatoes*

Lot No.	Treatment	Description	Catalase, cc. O <sub>2</sub> in 1 min.		Peroxidase				Methylene Blue Reduction Min.		Indophenol cc. Absorbed		0.01 N Iodine Absorbed	
			Treated	Check	Purpurogallin		Nadi method		Treated	Check	Treated	Check	Treated	Check
					Treated	Check	Treated	Check						
148 to 151	Cut tubers, soaked 1 hour in 1 percent NaSCN, Cobbler	Seed pieces with eyes	13.0	5.1	2.66	1.00	1.51	1.00	Sl.	Neg.	6.0	3.5	0.35	0.25
		Seed pieces without eyes	12.8	8.8	3.12	2.56	1.94	1.26	Pos.	Pos.	5.0	4.0	0.45	0.40
201 to 204	Ethylene chlorhydrin, cut tubers, dip method, 50 cc. per l., Cobbler	Seed pieces with eyes	19.5	7.5	4.45	1.00	1.29	1.00	0.25	Neg.	36.0	2.0	4.30	0.65
		Seed pieces without eyes	17.5	12.9	3.74	2.23	1.37	1.04	0.25	Neg.	37.0	2.5	4.25	0.75

other and with two other lots, with and without eyes, which received no treatment at all. The results are shown in table 13. The same changes that were induced in the lots having eyes were also found to have occurred in the lots without eyes. The differences between treated and check were not as great when the eyes were absent for the reason that the check lots without eyes gave in general higher readings than check lots with eyes. This may be due to the larger amount of cut surface which was exposed by the removal of the eye from the seed-piece, or to a wound effect from cutting, or both.

### **Relation of Percentage of Sprouting to the Results of the Tests on the Potato Juices**

In tables 1, 3, and 6, column 4 are given the percentage germination of the lots receiving the various chemical treatments. In the sense that the gradation of percentage germinations corresponded both to the series of chemical treatments and to the results of the enzym tests made upon the juices obtained from the potatoes, it may be said that a general relation was found. Certainly the most favorable concentrations of chemicals for inducing sprouting also gave the largest values in catalase, peroxidase, and other properties tested for. But the correlation can not be regarded as a very close one when the results are examined in detail. Thus, the thiourea responses were only small in many respects and practically zero for the catalase in the tissue exclusive of eyes, and yet the sprouting response was good. The failure of the sodium thiocyanate and thiourea treatments to furnish juices with the high capacity to reduce methylene blue such as was characteristic of the ethylene chlorhydrin treatments, when viewed in relation to the evident ability of these treatments to induce favorable sprouting, indicates that no close quantitative connection exists between methylene blue reduction capacity and sprouting. Even in the case of the ethylene chlorhydrin treatments, the whole-tuber treatments showed greater differences between treated and check lots than the cut-tuber treatments did, but the whole-tuber treatments were less effective in inducing germination. No single test could be used as an indicator of the capacity of a given lot of potatoes to sprout when planted; it is only when sprouting response is compared with the tests as a whole, that the general relation between increased enzym activity and high percentage sprouting becomes evident.

### **DISCUSSION**

It is important to note that the increases in enzym activity that were found to result from the treatment of potatoes with chemicals were not direct effects of the chemicals upon the enzymes themselves. In no case were any of the chemicals capable of increasing appreciably the enzym activity of the press-juice; they could cause depression if the concentration of the added chemical was high enough, but within the range of concentra-

tion at which the chemical could likely exist in the juice, no increase in activity was observed. So far as these enzymes were concerned the chemicals did not act by stimulating enzym action; it would be better to say that they induced the living matter to produce larger amounts of (or more active) enzymes.

An interesting feature of the experiments is the result showing that chemical treatment increased the enzym activity of potato tissue containing no buds. The tissue in these cases consisted largely of pith cells which are incapable of further growth except to form cork layers after injury by cutting or bruising. And yet these cells responded readily to the chemical treatments as is shown by the increased enzym action of the press-juice obtained from them. It is true that, on the whole, larger changes and quicker responses were found in the eye-tissue, but to what extent the changes in the tissue at some distance from the bud influenced the enzym activity at the eye or even the capacity of the bud to start into growth is not shown by these experiments, and is a problem that needs further work.

The effect of the ethylene chlorhydrin treatments in causing a change in the pH value of the juices (in the direction of reduced acidity) was very striking, especially when the whole tubers were exposed to the vapors of the chemical. On letting the tubers stand several days the acidity shift amounted in some cases to approximately a whole pH unit which is a ten-fold change in hydrogen-ion concentration. In the dip-method the shift was about 0.5 of a pH unit. A direct effect of ethylene chlorhydrin itself in causing this change in acidity can not be important here since an aqueous solution of ethylene chlorhydrin is acid, not alkaline, and furthermore the amounts of ethylene chlorhydrin absorbed by the tissue are too small to be effective in altering the pH in either direction. It would be interesting to know what changes within the tissue were responsible for this alteration of the pH. Potato juice is well buffered and the internal changes must have been extensive in order to give this result. Since the pH and buffer value of plant juices are influenced to a large extent by the content of organic acids and phosphates, quantitative measurements of these substances, together with the amounts of soluble cations, are desirable in connection with this problem.

We are aware of the possibility that the pH measurements may have been influenced by the increased reducing capacity of the juice and that the actual change in hydrogen-ion concentration may not have been so great as the data indicate. This point needs further investigation. But in the cases where the electrometric measurements showed a large reduction in acidity merely applying indicators to the surface of the tissue also showed a large shift in the alkaline direction.

We make no claim that the changes in enzym activity which are here reported are to be looked upon as the causes of the growth of buds or as furnishing proof as to the causes of the previous dormancy. They constitute

the measurements which have been made on the changes of the internal conditions which follow the treatment of the potato with chemicals. They are correlated with the initiation of growth processes and our data represent the result of an effort to push the measurements back to as early a stage of development as possible. It will be remembered that some of the evidence as to the initiation of change reached back to the first 24 hour period after treatment. It is clear, however, that the effect upon the living matter must have been produced at even an earlier hour, that the effect was first upon the living matter which was induced to begin activity, and which then brought about the changes that could be measured.

### SUMMARY

1. This is a report of experiments on the enzym activities of juices obtained from dormant potatoes that had been treated with chemicals which break the rest period, the measurements being made after the treatment but before visible sprouts appeared.

2. The effects of three chemicals were studied: ethylene chlorhydrin ( $\text{ClCH}_2\text{CH}_2\text{OH}$ ), sodium thiocyanate ( $\text{NaSCN}$ ), and thiourea ( $\text{NH}_2\text{CSNH}_2$ ). Although these three are different in chemical character they all break the dormancy of freshly harvested potatoes.

3. Increases in catalase, peroxidase, and the reducing properties of the juice as measured by the reduction of methylene blue, indophenol, iodine, and phosphotungstic reagents were observed. The increases were more marked in the case of the ethylene chlorhydrin treatments than for the other chemicals.

4. Increases in catalase and peroxidase began within about 24 hours after the end of the treatment with ethylene chlorhydrin, but the response to sodium thiocyanate and thiourea was less marked and occurred less quickly.

5. The increases in enzym activity were not direct effects of the chemicals upon the enzymes. In no case could the enzym activity of the press-juice be increased by the addition of the chemical to the juice. The chemical effect was indirect and was brought about by the action of the chemical upon the potato and not upon the enzymes in its juice.

6. In almost all cases when the potatoes were treated with different amounts of a chemical arranged in a descending series with respect to concentration, the juices obtained from these lots at a later period also showed a series of enzym readings corresponding to the series of concentrations of chemicals originally applied to the potatoes. This was not true, however, of the catalase readings in the thiocyanate treatments until after the press-juice had been dialyzed. The juice from thiocyanate-treated potatoes contained appreciable amounts of thiocyanate which, as shown by separate experiments, has a retarding effect upon catalase. A short period of dialysis allowed a separation of the thiocyanate from the enzym,

after which the catalase values of the thiocyanate-treated lots were higher than the checks, and gave a series of readings corresponding to the concentrations of chemicals used in treating the potatoes.

7. Ethylene chlorhydrin treatments induced a change in the pH of the juice in the direction of decreased acidity; and the amount of change in pH was related to the concentration of chemical applied in the treatment of the tissue. Only small changes in pH resulted from the sodium thiocyanate and thiourea treatments.

8. The enzyme changes were greater in the eye-tissue, and in most cases started sooner there than in the rest of the seed-piece. But treatments of potato pieces having no eyes showed that the same changes in enzyme activity occurred as in pieces containing eyes, the amount of the change being merely somewhat less. Presence of eyes was not necessary in the enzyme responses of tissue.

9. There was a general relation between the sprouting response and the enzyme measurements, since the treatments which induced good sprouting were also effective in causing the potatoes to furnish a juice of high enzyme activity. The correlation between enzyme activity and sprouting was not found to be close, however, when the data were examined in detail. Thus, the sodium thiocyanate and thiourea treatments were much less effective in increasing enzyme activity than would have been expected on the basis of the favorable sprouting response. And the enzyme activity of juices was greater from whole-tuber than from cut-tuber treatments in the case of ethylene chlorhydrin, although the cut-tuber method gives the better response in sprouting.

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## SOME EFFECTS OF ARTIFICIAL CLIMATES ON THE GROWTH AND CHEMICAL COMPO- SITION OF PLANTS<sup>1</sup>

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### INTRODUCTION

Plants growing under natural conditions are affected more or less by temperature, rainfall, humidity and carbon dioxide concentration of the air, light intensity, light quality, and length of day, as well as by many soil factors. In a study of the effects of these factors on plant growth it is obviously of great advantage to have as many factors as possible under close control. One or more may then be varied in a definite direction and the effect observed on growth, flowering, dry weight increase, chemical composition, or other measurable quantity associated with the development of the plant. Unless all factors are controlled any attempt to assign measured variations in a single factor as the causative agent of a particular development of the plant would seem to be mainly speculative. Yet many of the effects on plants of variation in environmental factors are so outstanding that even with poorly controlled environmental conditions certain factors have been without doubt correctly assigned as causative agents. Such factors as temperature and light intensity, especially when decreased greatly, have such marked effects on plant development that these factors were long ago assigned as causative agents of certain growth characteristics in plants. Length of day as a causative agent in initiating flowering has more recently been separated from two closely associated factors, temperature and light intensity. By an accurate control of day length when light intensity and temperature are high, Garner and Allard (4) have shown that day length alone determines flowering in some plants. Other causative agents which produce different developmental characteristics in plants no doubt exist and will be found when natural environmental factors can be controlled with more precision.

This series of experiments includes studies made with several species of plants growing in artificial climates. The plants were grown in some experiments with artificial light only as the source of energy for photosynthesis. In other experiments they were grown with daylight supplemented by artificial light for a period of six to 12 hours each night during

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

the months of February, March, and April. An attempt was made to grow plants throughout their life history with photosynthesis at or near its maximum rate by supplying a high light intensity and long day along with increased carbon dioxid concentration and a relatively high temperature. The effect of length of day on certain species was also studied in various combinations of temperature and carbon dioxid supply. Only a few combinations of various environmental factors were tested on the various species during these experiments. Some natural climatic factors also were found to be difficult to reproduce in an artificial climate. This is especially true of sunlight. The studies therefore are mainly a preliminary survey of the effect on plant development of a number of climatic factors reproduced as accurately as possible in an artificial climate. As more efficient light sources are developed it will be possible to approximate more closely a natural environment.

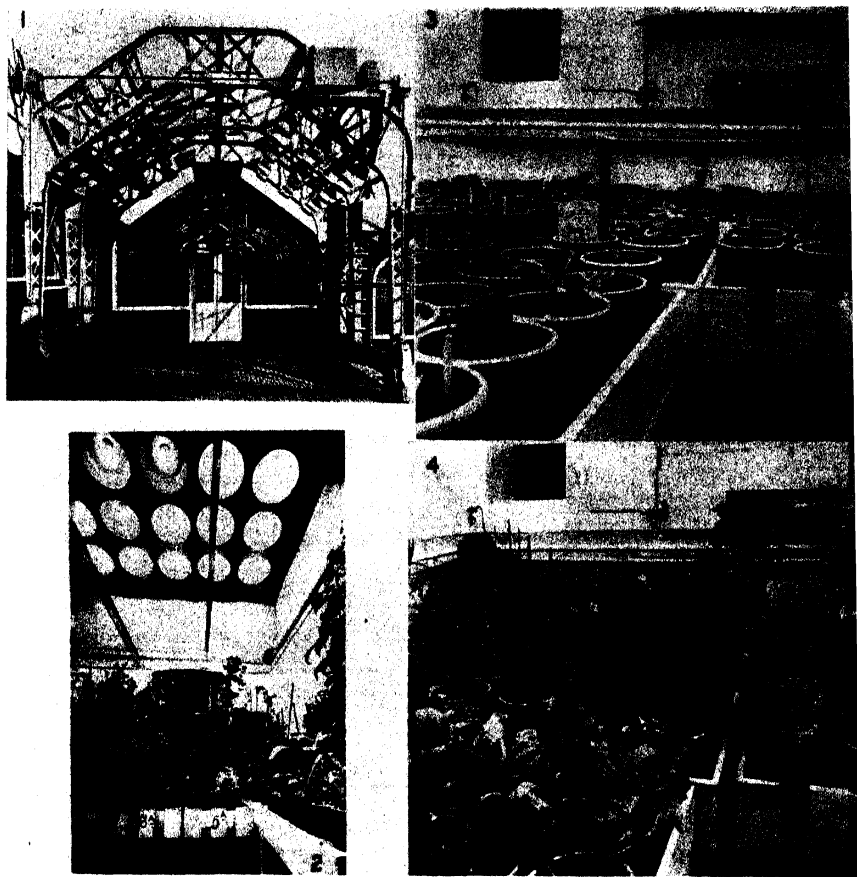
Chemical analyses of many plants grown under the different conditions are given together with a discussion of the effect of various factors on percentage carbohydrate and nitrogen in various tissues.

#### APPARATUS AND EQUIPMENT

Two kinds of equipment were used in these experiments. The first was the gantry crane greenhouses where sunlight was used during the day and a battery of 48 1000-watt lamps carried on the crane supplemented daylight for six to 12 hours each night. A photograph of the crane is reproduced in text figure 1. The second type of equipment was the constant condition rooms where plants were grown entirely with artificial light furnished mainly by a battery of 25 1000-watt lamps. The constant-light room is illustrated in text figure 2. A more detailed description of each of the two types of equipment follows.

#### Gantry Crane Greenhouses

The gantry crane greenhouses consisted of a set of two houses each  $26 \times 20$  feet placed end to end with a four-foot vestibule between. The vestibule was ventilated independently and served to check the diffusion of carbon dioxid into the house having only the normal concentration of this gas. Each house contained two benches running parallel to the ridge and arranged to support jars of growing plants at a height of 56 inches above the floor level. Each bench was approximately  $6\frac{1}{2} \times 20$  feet. They were spaced 30 inches apart so as to leave an aisle of this width running through the center of each house parallel to the ridge. The crane was built to travel upon iron rails placed on either side of the two greenhouses. It was driven into place over one of the greenhouses by means of an electric motor, where it remained for a period of six to 12 hours each night. It was removed each morning so as to avoid shading. The crane carried 48 1000-watt incandescent filament lamps arranged in rows so that



TEXT FIG. 1. Gantry crane carrying 48 1000-watt lamps used as a light source in the greenhouse to supplement daylight for 6 to 12 hours each night. TEXT FIG. 2. Plants growing with artificial light only in the constant-light room. This picture shows the position of the glass-water filter between the lamps and the plants. 25 1500-watt lamps were used as a light source. TEXT FIG. 3. A photograph of plants growing in the constant-light room four days after the start of the 1925 experiment. TEXT FIG. 4. Same as FIG. 3 except taken 17 days later. The lettuce plant on Chart 6 B (19 hour day) was grown in 17 days from the small plant similarly located in FIG. 3. The buckwheat plants on a 24 hour day in the rear of the date card grew to a height of 24 inches in 17 days. These plants were about 2 inches high in FIG. 3.

each half of the greenhouse roof was illuminated by 24 1000-watt lamps. The lamps were fitted with mirror reflectors and were arranged to distribute the light uniformly over each of the two benches with a minimum intensity over the walks in the center of the house. In the first experiments, 1924 and 1925, lamps were used with a rated voltage of 115 to 120. The current supplied was 108 volts at the main switchboard but there was a voltage drop toward the socket so that the measured voltage at the socket was only 105. In later experiments the rated voltage of the lamps was 105. This results in a greatly increased intensity and efficiency of the lamps. All lamps were replaced at the end of each experiment and it was found that a lamp with a rated voltage approximately identical with the socket voltage would maintain well the initial light output during the time of the experiment. Experiments in general lasted 8 to 10 weeks, during which time the lamps burned a total of 700 to 850 hours. It should be pointed out that these lamps are designed to be burned for 1000 hours and that unless current is very cheap it does not pay to burn them longer since the light output falls rapidly after this life span has been reached.

In the first experiments air temperature in the gantry crane houses was controlled by thermostats operating steam control valves by means of compressed air. These were not dependable unless supplemented by hourly inspection, and they were later replaced by electrically operated solenoid valves. This gave a very positive control of the steam supply for heating and by hourly inspection during warm weather the greenhouse vents could be kept open far enough to keep the temperature down. Temperatures were recorded continuously during all experiments by means of recording galvanometers connected with electrical resistance thermometers placed in each house. In general, temperatures were held within a plus or minus variation of approximately three degrees.

The control plants were kept in a greenhouse similar in dimensions to the gantry crane houses. Here, however, a slightly better control of air temperature and humidity was obtained by recirculating the air by means of a standard air-conditioning system. The operation of this system depends upon first, the saturation of the air coming from the greenhouse with a thermostatically controlled mixture of cold water and steam; and second, the re-heating of the air in the ducts as it rises toward the greenhouse to bring it to the desired temperature. Depending upon the temperature of saturation and the temperature of re-heating a definite degree of relative humidity can be maintained within certain limits. In most of the experiments reported herewith the thermostats were set to maintain a relative humidity of 80 percent.

### Constant Condition Rooms

These rooms are equipped for growing plants entirely with artificial light. They are two in number, the constant-light room and the adjoining dark room. Each room is approximately 11 feet square and is located in

the basement under the greenhouses. The rooms are connected by a short closed corridor, and in order to obtain different lengths of day and night plants may be moved from the light to the dark and back again with no temperature change and the minimum of shock. The same air was re-circulated through both rooms by means of standard air-conditioning machinery similar to that already described in connection with the control greenhouses. The only difference was that ice water supplied by a 15-ton ice machine was used, while the amount of cooling in case of the control greenhouse was limited to the temperature of tap water. Air temperature in the light and dark room was maintained accurately within a plus or minus range of one degree centigrade, and humidity controlled to a plus or minus three percent relative during the whole growth period of the plants. Wet and dry bulb temperatures were recorded by means of recording galvanometers in connection with resistance thermometers.

The main light source in the constant-light room was from 25 1500-watt gas-filled tungsten filament lamps suspended from a metal frame in the ceiling of the room. The lamps were fitted with Reflectors and Light Manufacturers Standard Reflectors and were arranged to give uniform illumination on the benches where the plants were grown. A false ceiling of clear plate glass was built between the lamps and the growing plants. This was fitted with a weir at one end which was adjustable in height. Water was fed in at the opposite end and the weir was set to maintain a layer of water  $\frac{1}{4}$  to  $\frac{1}{2}$  inch in thickness over the entire surface of the glass plate. This glass-water filter served to absorb some of the infra-red output of the lamps. It also resulted in a considerable loss of energy in the visible region. This was especially true when dust and green algae were allowed to accumulate. Algae gave a great amount of trouble since such conditions of high temperature and high light intensity are ideal for their growth. It was found that a cheesecloth bag filled with zinc oxid and suspended in the water supply served to check the growth of algae. This treatment supplemented by a thorough scrubbing of the glass surface every other day maintained fairly well the initial transmission of the glass-water filter.

A motor driven fan was used to supply forced ventilation to the space around the lamps and above the glass-water filter. This also served to get rid of the large amount of excess heat from the lamps. This fan motor switch was interlocked with the switches supplying power to the lamps so that the lamps could not be burned until the fan motor was running.

In the first experiment in 1924 the glass-water filter was 40 inches from the tips of the lamps. The distance from the filter to the soil in which the plants grew was approximately 65 inches, making a total distance from the lamp to the soil of about 105 inches. In later experiments the lamps were moved down toward the filter so that in the last experiments (1926 and 1927) the lamps were only 19 inches above the surface of the water, or 84 inches above the soil. Since plant growth was very rapid the distance between

the tip of the plant and the filter rapidly decreased with a resulting very slight increase in light intensity. Some of the taller plants such as sunflower, corn, and buckwheat grew sufficiently to touch the filter before the experiment was closed.

The resulting light on the soil in which the plants were grown was measured by both the Macbeth Illuminometer and a pyrheliometer described by Kimball and Hobbs (10) and in use at various Weather Bureau stations for recording solar intensity. These instruments will be discussed later. The pyrheliometer was connected with a recording millivoltmeter and calibrated in energy units. These data are also tabulated later. In the first experiment of 1924 light intensity decreased until at the end of the experiment it had fallen to about 50 percent of the original value. This was found to be due to the aging of the lamps which usually occurs after about 40 days of continuous burning. In all later experiments lamps were replaced or the experiment discontinued after 45 days.

### Carbon Dioxid Supply

In one of the gantry crane houses and in the constant-light room the carbon dioxid concentration was maintained at ten times the normal, or about 0.3 percent. The carbon dioxid was supplied from steel cylinders holding 50 pounds of this gas. Three cylinders were connected to a manifold at the same time. The gas was first heated as it left the tanks by means of a coiled tube immersed in hot water. This treatment prevented freezing which would otherwise occur due to the rapid expansion of the gas. It was then expanded through a reducing valve into a 30-gallon cushion tank which insured an even flow through the gas meters located in the lines which delivered the gas to the growing houses. These meters were identical with the household type which public service companies use to meter illuminating gas except that they were fitted with a larger dial for more accurate reading. After a few determinations by gas analysis of the number of revolutions per minute necessary to maintain the concentration desired the valves were opened so as just to maintain this rate on the meters. In the 1926 and 1927 experiments a low range carbon dioxid recorder made by the Leeds and Northrup Company was used. This recorder had a range of 0 to 3.5 percent. It has been described by Rosecrans (17).

Approximately six cylinders of carbon dioxid or 300 pounds of the gas were required in each 24-hour period to maintain a concentration of about 0.3 percent in one gantry crane house and in the constant-light room. When the greenhouse vents at both the ridge and eaves were opened to keep temperature down even this rapid flow of gas failed to maintain the desired concentration. It should be stated here that it is almost impossible to maintain any appreciable concentration of carbon dioxid in a greenhouse in this way on account of very rapid convection currents which sweep the gas out as fast as it is delivered.

A separate experiment was made in 1926 to determine whether one percent carbon dioxid would produce more growth than 0.3 percent during the months of October, November, and December without additional light. It was found that a great number of plants produced more weight when grown in 0.3 percent concentration of this gas but that one percent gave no further increase. The higher concentration produced no injury, but it was much more expensive and difficult to maintain so that the lower concentration of 0.3 percent was used in all other experiments.

In 1926 and 1927 a method for producing carbon dioxid from flue gases was studied. In the results of experiments presented here data concerning plants grown with carbon dioxid from this source are listed under the caption "Flue gas" or sometimes in the case of photographs, "F.G." Gases arising from the burning of anthracite coal were treated in the following way. Hot gases were pumped by means of a negative pressure fan from the stack immediately above the boiler, first through a steel scrubbing tower where they were atomized with water. The gases were cooled and partially washed in this tower. They passed on through a similar tower where they were atomized with a one percent potassium permanganate solution. The gases then passed into a third tower where they were washed again with water and thoroughly saturated before moving through four filter cabinets. The first three filter cabinets were filled with trays of sawdust and sphagnum moss while the last one contained trays of glass wool which removed the last traces of finely divided carbon. The function of the permanganate solution is not definitely known. It has been found by trial, however, that it oxidizes certain compounds in the gases which are otherwise harmful to plants. It is slowly oxidized so that the solution becomes completely decolorized in about three to four weeks when the apparatus is running continuously for nine hours each day. The permanganate solution returning from the tower is collected in a 300-gallon earthenware jar and re-circulated by means of a small pump. The third tower serves to wash out any permanganate carried over and effectively increases the size of the finely divided soot particles so that all traces of soot are removed by the filters. This is especially important when the gases are used in greenhouses since any finely divided carbon brought in by the gases is precipitated on the cold surface of the glass as well as on plants and in time builds up a sooty, black deposit. The present filtering arrangement has been used to produce gas for a single greenhouse during two seasons of growth in the winter months and has caused no appreciable blackening of the glass or white framework during this time.

### Light Intensity Measurements

During the 1924 experiments light intensity was measured only by a Macbeth illuminometer. In all later series both the illuminometer and a recording thermo-electric pyrheliometer were used. The former instrument



is a portable photometer carrying a lamp which is calibrated so as to give a known illumination value with a definite current. It is especially useful in measuring the illumination of artificial light sources indoors. On account of the color difference between the artificial light source in the instrument and sunlight it is more difficult to measure solar intensities. There is also no relation between the reading of an illuminometer in foot candles and the radiant energy received in gram calory units which is applicable to all light sources since the illuminometer is concerned only with visible radiation. A factor can be obtained, however, for a given artificial light source operating under known conditions which will allow the conversion of the reading of an illuminometer in foot candles to energy units as determined by a pyrheliometer or other apparatus for measuring radiant energy. A 1000-watt lamp fitted with a standard reflector at 37.5 inches gave an illumination value of 620 foot candles as measured by an illuminometer or 0.3 of a gram calory per square centimeter per minute as measured by the pyrheliometer. The factor in this case is approximately 2100. This factor is also approximately the same as that obtained in the gantry crane houses. Ageing of the lamps decreases the value while the effect of a glass water screen as used in the constant-light room is to increase it greatly due to absorption of the infra-red region with very little loss in illumination value. In the case of the constant-light room the factor is approximately 5000, except in 1925 when the line voltage was too low to operate the lamps efficiently. Kimball (9) has found a similar illumination equivalent for sunlight which gives the illumination value in foot candles from the energy value as determined by the pyrheliometer, this value being 6700 for cloudless skies and 7000 for a sky covered with clouds. The value as determined in the constant-light room approaches that for sunlight. While this factor approaches the value 6700 found by Kimball for solar radiation it does not indicate that the light in the case of the constant-light room was equal to solar radiation in spectral energy distribution. The approximate spectral radiation components of four different light sources similar to those used in this work as published by Coblentz, Dorcas, and Hughes (3) are as follows:

	In percentage of the total radiation to 12,000 ft.		
	450-600 m $\mu$	600-1400 m $\mu$	1400-4200 m $\mu$
Sun, Washington, D. C. ....	22.0 m $\mu$	39.5 m $\mu$	19.7 m $\mu$
Air mass 1.3, July 28, 1926, 11 A.M. ....	6.7 "	3.2 "	20.5 "
Quartz mercury arc. ....	3.8 "	29.8 "	54.2 "
Gas filled tungsten lamp, 12.7 amps. ....	9.8 "	19.5 "	39.2 "
White flame carbon arc 30 amps. ....			

#### COLLECTION AND TABULATION OF DATA

Weekly measurements of growth in height were made and any development of buds, flowers, and fruit or changes in foliage color were noted.

These measurements were supplemented by frequent photographs, several of which are reproduced along with the chemical data.

### Chemical Analysis <sup>2</sup>

Many plants grown under the various conditions were sampled and analyzed for various carbohydrate and nitrogen constituents. The sampling was usually done toward the end of an experiment when the plants were nearing maturity except in certain cases where an attempt was being made to determine the effect of age on these constituents. Sampling was done at the end of the period of exposure to light in every case except where otherwise noted. In the case of those plants growing in the gantry crane greenhouses which were exposed to daylight plus artificial light from 6 P.M. until midnight, the plants were harvested at midnight and placed in the cold room at a temperature near zero degrees Fahrenheit where they remained until they were ground and preserved in alcohol on the following day. Plants could be kept at this temperature for 24 to 48 hours with very little change in carbohydrate fractions. Because of the difficulty in getting plant roots free from soil only the aerial portion in general was sampled. There were a few exceptions to this procedure in the case of those plants having large fleshy roots. The tissue was ground with a Russwin steel knife mill and divided into duplicate or triplicate samples for analysis. Separate samples were also taken for moisture determinations. The samples for analysis were placed in 300-cc. Erlenmeyer flasks and weighed. One-tenth gram of calcium carbonate was added and the flasks were filled two-thirds full of boiling 95-percent alcohol and boiled for ten minutes. They were then filled to the neck with alcohol and stoppered and placed in storage until the tissue could be conveniently analyzed.

In general the official methods for feeding stuffs published by the American Association of Official Agricultural Chemists was followed. The tissue was extracted with 50-percent alcohol. Reducing sugars were determined on the cleared extract and the fraction listed as sucrose determined by reduction, after hydrolysis of this extract, by subtracting the value for reducing sugars first obtained. The acid hydrolyzable fraction was determined as reducing substances in the hydrolyzed and cleared residue. Hydrolysis was accomplished by heating for two and one-half hours with 20 cc. of hydrochloric acid, specific gravity 1.125, and 200 cc. of water in a flask fitted with a reflux condenser. The results are calculated as dextrose and multiplied by the factor .9.

Soluble nitrogen was determined from an aliquot of the alcoholic extract and insoluble nitrogen from an aliquot of the dried residue by the Kjeldahl method modified to include nitrates.

The amount of moisture was determined by drying duplicate samples of the ground tissue in a vacuum oven at 70° C.

<sup>2</sup> The writers are indebted to Dr. J. E. Webster for analyses of plants grown in the 1924 series.

TABLE 1. *Effect on Chemical Constitution of Keeping Tomato Plants in Darkness for Various Periods Before Sampling. Whole Aerial Portion*

Growth Conditions	Weight per Plant (Grams)	Moisture %	Nitrogen, % Dry Weight		Carbohydrate, % Dry Weight				Carbohydrate Nitrogen
			Soluble	Total	Acid Hydro- lyzable	Sucrose	Dextrose	Total	
1. Exposed July 6 to 30, 1928, to sunlight. Leaves only									
1. Greenhouse, sampled at 3 P.M.	15	83.0	.24	2.42	33.7	1.41	2.94	38.1	15.8
2. Same except after 17 hours in darkness.	16	84.2	.25	2.53	29.9	.57	1.71	32.2	12.8
3. Grown outdoors, sampled at 3 P.M.	10	82.7	.29	2.83	26.8	1.45	3.35	31.6	11.2
4. Same as 3 except after 17 hours in dark- ness.	10	84.3	.19	2.87	26.4	.57	2.04	29.0	10.5
Stems only									
5. Same as 1.	19	88.5	.26	1.13	15.0	4.52	5.83	25.4	22.4
6. Same as 2.	24	89.1	.18	1.01	14.3	2.84	6.06	23.2	23.0
7. Same as 3.	12	87.0	.23	1.08	13.3	4.38	6.62	24.3	22.5
8. Same as 4.	14	88.9	.27	1.17	14.5	2.61	6.22	23.3	19.5
2. Exposed to artificial light, 700 f.c. without filter April 30 to May 4 as compared to greenhouse plants. Leaves only									
9. Greenhouse, sampled at 3 P.M.	15	86.3	.29	3.19	23.9	1.49	2.00	27.4	8.6
10. Same except sampled in early A.M.	16	86.7	.30	3.58	17.5	.26	1.48	19.2	5.4
11. Artificial light, sampled at once.	15	85.7	.29	2.78	28.0	1.11	2.25	31.3	11.2
12. Same except sampled after 40 hours darkness.	16	88.9	.58	3.85	9.5	0.0	1.20	10.7	2.78
Stems only									
13. Same as 9.	26	91.7	.30	1.14	13.9	2.74	5.63	22.3	19.6
14. Same as 10.	22	91.3	.28	1.09	13.0	2.22	6.14	21.3	19.5
15. Same as 11.	30	91.4	.46	1.37	14.0	2.17	7.14	23.3	17.0
16. Same as 12.	34	92.4	.71	1.55	12.3	1.01	5.03	18.3	11.8

### Effect of Darkness on Carbohydrate Fractions

A series of analyses was made on tomato plants exposed in one case to daylight and in another to artificial light and then kept in darkness at room temperature for various periods before sampling. This study was made primarily to determine the magnitude of the decrease in carbohydrate fractions during short periods of darkness. These periods are comparable to the effect of night and periods of very low light intensity which plants often receive when growing under natural conditions. The data are given in table 1. It will be observed from the data given that plants gain slightly in percentage moisture during such periods of rest. Nitrogen fractions are not changed appreciably. Total carbohydrates in general decrease slightly after 17 hours of darkness while after 40 hours this value has decreased to about one-third of the original figure. The greatest decrease is in the leaves in the shorter periods of darkness, while in prolonged darkness even the stems are greatly depleted in easily available carbohydrates. There is a difference in the percentage carbohydrate in plants sampled in the evening after a day of rapid photosynthesis as compared with plants sampled in the morning before any photosynthesis takes place. The effects of these rapid changes in carbohydrates are reflected in the carbohydrate-nitrogen ratio as indicated in the last column of table 1. It is seen that darkness causes wide variations in this relation so that a combination of light and darkness can be chosen to produce in a few hours either a high or low carbohydrate plant depending upon the time the plant is sampled relative to periods of light and darkness. These changes are accomplished through an alteration of the percentage of carbohydrate in the whole plant with very little change in the percentage of nitrogen. The carbohydrate-nitrogen ratio will be discussed later in greater detail.

### Errors of Sampling

In order to gain some idea of the individual errors of sampling, two species used extensively in these studies were selected for a detailed study of sampling errors. One was the aerial portion of radish and the other the tomato plant. A large number of plants of each species were grown under similar conditions. They were then sampled in smaller separate groups. These data are included in tables 2 and 3. Table 3 shows both the variation in duplicate samples and that of individual plants. By comparing the figures in each column with the average at the foot of the respective columns it will be observed that the sampling errors are relatively small. Groups of individuals from both species of plants when grown under the same conditions vary but little in chemical constituents.

### CONDITIONS OF EXPERIMENTS

#### *Experiment 1. June 3 to August 15, 1924*

Constant-light room, artificial light only.

Temperature 78° F.

TABLE 2. *Analyses of Special Radish Tops Grown in Control. Seed Planted Feb. 12 and Plants Sampled March 8, 1927*

Sample	Total Wt. of Tops	Wt. of Tops per Plant	Moisture	Nitrogen						Acid Hy- drolyzable Material		Sucrose		Dextrose		Total Carbohy- drates	
				Insoluble		Soluble		Total		Green	Dry	Green	Dry	Green	Dry	Green	Dry
				Green	Dry	Green	Dry	Green	Dry								
R 1	82	3.0	92.38	0.30	3.95	0.15	1.97	0.45	5.92	0.53	6.99	0	0	0.14	1.82	0.67	8.81
R 2	89	3.1	92.48	0.29	3.81	0.16	2.19	0.45	6.00	0.57	7.54	0	0	0.15	2.04	0.72	9.58
R 3	77	2.8	91.47	0.30	3.49	0.17	2.03	0.47	5.52	0.52	6.15	trace	trace	0.16	1.83	0.68	7.98
R 4	63	2.1	92.23	0.28	3.60	0.17	2.27	0.45	5.87	0.54	6.95	0.10	1.33	trace	trace	0.64	8.28
R 5	59	2.2	92.38	0.28	3.68	0.15	1.92	0.43	5.60	0.58	7.60	0.23	2.95	trace	trace	0.81	10.55
R 6	50	1.6	90.63	0.38	4.07	0.23	2.43	0.61	6.50	0.64	6.86	0.16	1.68	0	0	0.80	8.54
R 7	69	1.9	91.85	0.29	3.55	0.14	1.75	0.43	5.30	0.57	7.00	0.24	2.99	trace	trace	0.81	10.00
R 8	64	2.0	91.25	0.34	3.84	0.21	1.23	0.55	5.07	0.61	6.96	0.07	0.81	0.11	1.23	0.79	9.00
R 9	64	2.2	91.13	0.34	3.85	0.22	2.48	0.56	6.33	0.60	6.74	0.17	1.93	trace	trace	0.77	8.67
R 10	80	2.8	91.70	0.31	3.75	0.15	1.84	0.46	5.59	0.60	7.21	0.15	1.82	0.12	1.40	0.87	10.43
R 11	71	2.4	92.24	0.24	3.08	0.16	2.03	0.40	5.11	0.57	7.38	0.12	1.52	0.08	1.09	0.77	9.99
Average			91.79	0.34	3.69	0.17	2.01	0.48	5.71	0.58	7.05	—	—	—	—	0.76	9.25

TABLE 3. *Duplicate Analyses of Tomato Plants to Show Individual Variation. Grown Under a Glass which Transmits Only 40 Percent of Sunlight. 1927. Whole Aerial Portion*

Sample	Total Wt. of Plant	Moisture	Nitrogen						Acid Hy- drolyzable Material		Sucrose		Dextrose		Total Carbohy- drates	
			Insoluble		Soluble		Total									
			Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry
			Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry
0224	472	91.80	0.180	2.21	0.148	1.82	0.328	4.03	0.872	10.70	0.146	1.79	0.288	3.53	1.346	16.02
		91.89	0.182	2.23	0.142	1.74	0.324	3.97	0.862	10.58	0.145	1.78	0.284	3.48	1.291	15.84
096	414	90.77	0.178	1.93	0.185	2.01	0.363	3.94	0.991	10.76	0.238	2.58	0.398	4.32	1.627	17.66
		90.80	0.178	1.93	0.193	2.10	0.371	4.03	0.966	10.48	0.256	2.78	0.382	4.15	1.604	17.41
0215	690	91.55	0.165	1.95	0.157	1.86	0.322	3.81	0.856	10.13	0.133	1.57	0.334	3.95	1.323	15.65
		91.53	0.169	2.00	0.161	1.90	0.339	3.90	0.881	10.40	0.149	1.76	0.319	3.77	1.349	15.93
093	958	92.53	0.165	2.21	0.127	1.70	0.292	3.91	0.805	10.81	0.120	1.66	0.165	2.21	1.090	14.63
		92.37	0.159	2.13	0.130	1.74	0.289	3.87	0.829	11.13	0.112	1.50	0.147	1.97	1.088	14.60
500	510	92.17	0.169	2.16	0.143	1.82	0.312	3.98	0.756	9.64	0.145	1.85	0.265	3.38	1.166	14.87
		92.15	0.163	2.08	0.147	1.88	0.310	3.96	0.798	10.18	0.137	1.75	0.239	3.05	1.174	14.98
0191	398	90.79	0.194	2.12	0.158	1.73	0.352	3.85	1.081	11.84	0.124	1.36	0.379	4.15	1.584	17.35
		90.95	0.198	2.17	0.156	1.71	0.354	3.88	0.957	10.48	0.170	1.86	0.340	3.72	1.467	16.06
1072	684	92.22	0.171	2.19	0.138	1.77	0.309	3.96	0.731	9.37	0.154	1.97	0.261	3.35	1.146	14.69
		92.17														
403	457	91.50	0.198	2.35	0.156	1.85	0.354	4.20	0.826	9.80	0.109	1.29	0.317	3.64	1.252	14.73
		91.63	0.192	2.28	0.135	1.60	0.327	3.88	0.808	9.58	0.133	1.58	0.293	3.48	1.234	14.64
Average		91.5	0.184	2.11	0.151	1.82	0.328	3.95	0.871	10.00	0.151	1.81	0.294	3.50	1.310	15.70

Humidity 80 percent.

Carbon dioxide concentration 0.1 to 0.8 percent; 4 50-pound tanks used in each 24 hours.

Light exposures of 5, 7, 12, 17, 19, and 24 hours in each 24 hour period.

Light source 25 1000-watt 120-volt lamps operating on 105-volt supply.

Light filter one-half inch of plate glass plus one inch of water.

Average Macbeth readings at soil level: 450 foot candles.

*Experiment 2. August 28 to October 15, 1924*

Same as Experiment 1 except no additional carbon dioxide.

Average Macbeth reading 350 foot candles.

*Experiment 3. February 28, to May 14, 1925*

1. Gantry crane House 1, daylight supplemented with artificial light from midnight until 6 A.M. each night, normal concentration of carbon dioxide. House 2, daylight supplemented with artificial light from 6 P.M. until midnight and with carbon dioxide concentration at about 0.3 percent or ten times the normal.

Light source 48 1500-watt 120-volt lamps operating on 105-volt current.

Average pyrreheliometer reading: 0.45 gram calory per square centimeter per minute.

Equivalent in foot candles: 819.

Temperature in all houses 78° F.

2. Constant-light room, artificial light only.

Carbon dioxide concentration about 0.3 percent.

Light source 25 1500-watt 120-volt lamps operating on 105-volt current.

Light filter of plate glass with water, average depth three-eighths inch.

Average pyrreheliometer reading: 0.3 gram calory per square centimeter per minute.

Macbeth reading: 800 foot candles.

Temperature 78° F.

Humidity 80 percent.

Light exposures of 5, 7, 12, 17, 19, and 24 hours in each 24 hour period.

*Experiment 4. February 28 to May 8, 1926*

1. Gantry crane houses same illumination as 1925 except 48 1000-watt 105-volt lamps operating on 105-volt current supply.

Average pyrreheliometer reading: 0.36 gram calory per square centimeter per minute.

Macbeth reading: 760 foot candles.

House 1, daylight plus gantry crane illumination from midnight until morning.

House 2, daylight plus gantry crane illumination from 6 P.M. until midnight and with ten times the normal carbon dioxide concentration.

Temperature 68° F. in all houses.

Humidity 80 percent in control house.

2. Constant-light room; artificial light only.

Carbon dioxid concentration ten times normal.

Light source 25 1500-watt 105-volt lamps operated on 104-volt current.

Light filter same as Experiment 3.

Four mercury vapor arcs in glass tubes.

Average pyrheliometer reading: 0.25 gram calory per square centimeter per minute.

Macbeth reading: 1200 foot candles.

Temperature 68° F.

Relative humidity 80 percent.

Light exposures of 5, 7, 12, 17, 19, and 24 hours in each 24 hour period.

*Experiment 5. January 28 to April 8, 1927*

1. Gantry crane houses same illumination as in 1926 except only one house, number 2, illuminated.

House 2, daylight 12 hours plus gantry crane 12 hours from 6 P.M. until 6 A.M. making a 24 hour day.

Carbon dioxid ten times normal.

Temperature 78° F. except in a small vestibule held at 68° F.

Relative humidity in control house 90 percent.

2. Constant-light room, artificial light only.

Carbon dioxid concentration ten times normal.

Light source 22 1500-watt 105-volt lamps operated on 105-volt current. Three 25 amperes white flame carbon arcs. Two mercury vapor arcs in glass tubes.

Light filter same as experiment 3.

Average pyrheliometer reading: 0.24.

Macbeth reading: 1400 foot candles.

Temperature 68° F.

Relative humidity 90 percent.

## RESULTS OF EXPERIMENTS

More than thirty different species of plants were grown in these experiments. It was found impossible to get a complete set of data on the whole 30 species as regards chemical analysis and day-length effects. A few representative species of special interest were therefore selected for a more detailed study. These have been grown with different day-lengths from five hours to 24 in the constant-light room and also with daylight supplemented by six to 12 hours each night with artificial light from the gantry crane.

Plants in general were found to increase greatly the weight of tissue produced when given daylight plus six hours additional light each night. A still further increase was produced by increasing carbon dioxid concen-



tration along with supplementary lighting. Many plants grew very rapidly in the constant-light room with artificial light only. Text figures 3 and 4 show 17 days' growth under these conditions. The large lettuce plant shown in the foreground of figure 4 was produced in this time period from the small plant similarly located in figure 3 and illustrates the rapid growth under these conditions. When grown with artificial light the weight of tissue produced increases with day length up to approximately an 18 hour day while there is no corresponding increase when given a 24 hour day. Some species were found to grow as well with continuous 24 hour illumination as on an 18 hour day, while others were found to be greatly injured by a 24 hour day. The results of the chemical analyses of various species as related to carbohydrate-nitrogen ratio, injury of long day, and specific effects of the various conditions on a number of plants will be discussed in the following pages.

### **Carbohydrate-nitrogen Content as Related to Flowering and Length of Day**

Considerable study has been made of the effect of various day lengths on the carbohydrate-nitrogen relations of various plants grown in this series of experiments. The study has been especially directed toward representative varieties of each type of plant as originally worked out by Garner and Allard (4), the short and long day and the everblooming types. Both radishes and lettuce are of the long day type and both grow well up to a 24 hour day or continuous illumination. They were therefore selected as suitable plants for growing on a number of day lengths from five to 24 hours. Similarly, salvia was selected as a typical short day plant and buckwheat as an everblooming type. Salvia flowers well on day lengths of 15 hours or shorter while buckwheat flowers equally well on all day lengths from five hours to 24. In this series of experiments it was hoped to establish a possible relation between the percentage composition of carbohydrate and nitrogen constituents and the day length at which the plants would flower. Assuming that day length determines flowering in these species through a building up of a certain amount of carbohydrate with relation to the total amount of nitrogen, one should be able to determine how much of each fraction is necessary before flowering is initiated in the long day plant and what carbohydrate maximum just prevents flowering in the short day plant. This premise also assumes that the amount of photosynthetic material manufactured is in some simple proportion to the dosage of light, that is an intensity  $\times$  the time of exposure, within certain limits. Where more carbohydrates are built up with greater dosage of light it is reasonable to expect that translocation and further synthesis will not be increased at the same rate, consequently carbohydrates will increase to a greater or lesser degree, with increasing day length, if intensity remains the same. If the ratio of total carbohydrate to total nitrogen is effective in determining flowering it should be possible to regulate this process



TEXT FIG. 5. Lettuce plants grown on 5, 7, 12, 17, 19, and 24 hour days in the constant-light room, showing the flowering on day lengths of 17, 19, and 24 hours. TEXT FIG. 6. *Salvia* plants flowering on short day lengths of 5, 7, and 12 hours. Only an occasional terminal flower is produced on a 17 hour day. This plant flowers well on all day lengths from 5 to 15 hours. TEXT FIG. 7. Buckwheat plants grown in constant-light room. This plant flowers on all day lengths from 5 to 24 hours. The height growth increases with day length up to 19 or 24 hours. The control plants were grown in the greenhouse. The plants are all 32 days old from seed. TEXT FIG. 8. Tomato plants from constant-light room grown on 5, 7, 12, 17, 19, and 24 hour days. The control plant was grown in a greenhouse. The photograph shows the extreme injury of continuous illumination on tomato plants.

through the increase or decrease of nitrate in the soil providing the plant has no regulatory mechanism which limits the absorption of this salt. It should also be possible to initiate or inhibit flowering by adjusting light intensity in combination with suitable day length and in this way regulate the ratio through an increase or decrease of carbohydrate, providing again that the plant has no regulatory mechanism for maintaining only a certain amount of carbohydrate reserve. In previous work it has been observed that certain plants such as corn are able to regulate the amount of nitrate taken in so that it is difficult to induce this plant to take up enough nitrate to increase the total percentage of nitrogen in the plant. The most promising method of changing the carbohydrate-nitrogen balance therefore seemed to be an increase of light intensity in combination with suitable day lengths. Both the decrease and increase of nitrate in the soil as well as different light intensities in combination with various day lengths have been used in this study. The analytical data are reported in tables 4 to 9.

In the case of long and short day plants the tables are divided into two parts, those which either were flowering or had the flowering response, and those which were not flowering or had no flowering response. Flowering response is here used to mean the ability of a long day plant to flower after it has been kept for a time on a long day and then transferred to a short day where it will later flower. This is a common characteristic of both radishes and lettuce. The opposite situation also exists; that of a short day plant flowering on a long day after it has been transferred, but this has not been studied carefully in the work reported herewith.

The "dose" of light necessary to initiate flowering in long-day plants growing in greenhouses during the short days of winter involved mainly the length of day (photographs of long and short day and "everblooming" types are shown in text figs. 5, 6 and 7). Intensity was not a factor since the lowest intensity used (170 foot candles) was well above the minimum for initiating flowering. While this exact minimum has not been accurately determined it is known to be very low as compared with sunlight. Light diffusing from the gantry crane greenhouse in these experiments induced flowering in radish and lettuce in another greenhouse at an illumination value of about five foot candles. Intensities of this order produce little or no weight increase in plants, and are probably well below the minimum for survival.

The total carbohydrate and total nitrogen in percent of dry weight for radish and lettuce together with the ratio of the two are listed in tables 4 and 5. The great variation of these fractions in plants grown under various conditions is shown. Total carbohydrate in radish (table 4) varies from 7.47 to 34.73 percent among the plants which were flowering or showed flowering response, while there was a similar range, 8.95 to 21.23 percent, among those which did not flower. Total nitrogen varied from 1.51 to 5.92 percent in the first case and from 2.77 to 7.27 percent in the second.

TABLE 4. *Carbohydrate-nitrogen Relation in Radish, a Long Day Plant. Plants Grown With Various Lengths of Day. Whole Aerial Portion*

Treatment of Plant, Age, and Number of Days in Growth Condition	Total Carbo- hydrate, % Dry Weight	Total Ni- trogen, % Dry Weight	Carbohydrate Nitrogen
1. Plants flowering or showing flowering response when transferred to short day. 1927 Series unless otherwise indicated			
Control greenhouse, 51 days (1), F.	9.67	4.91	2.0
Greenhouse 2, 21 days, F.S.D.	13.14	5.75	2.6
“ “ 2, 41 days, F.	19.94	3.77	5.3
15 hour day, 52 days, F.	15.41	3.36	4.6
17 “ “ 45 days, F.	9.59	2.45	4.1
19 “ “ 20 days, F.S.D.	21.90	4.67	4.7
19 “ “ 40 days, F.	21.90	2.87	7.6
24 “ “ 29 days, F.S.D.	25.47	4.68	5.4
24 “ “ 35 days, F.	27.53	3.45	8.0
24 “ “ 31 days, F.	17.15	4.00	4.3
24 “ “ 24-hour night, 14 days, F.	10.19	5.63	1.9
Greenhouse + 8 hours artificial light 170 f.c. 15 days, F.S.D. (2)	7.47	5.92	1.3
Greenhouse + 8 hours artificial light 170 f.c. 44 days, F. (2)	7.87	5.72	1.4
Greenhouse 1, 46 days, 1926 F.—grown in sand	27.44	1.51	18.1
Greenhouse 2, 46 days, 1926 F.—grown in sand	34.73	1.52	22.8
2. Plants not flowering and having no flowering response			
Greenhouse control, 32 days (4)	12.69	5.99	2.1
“ “ 51 days	18.50	7.27	2.6
7 hour day—40 days	10.55	5.88	1.8
7 “ “ 54 “	8.95	6.33	1.4
12 “ “ 20 “	19.14	5.70	3.4
12 “ “ 61 “	8.89	5.23	1.7
15 “ “ 20 “ (3)	17.67	4.69	3.8
17 “ “ 20 “ (3)	21.12	4.84	4.4
Greenhouse + 8 hours artificial light 170 f.c. 7 days (age 31 days)	12.34	5.98	2.1
Control greenhouse—31 days	10.70	5.72	1.9
12 hour day—12 hour night—14 days	10.16	5.22	2.0
Greenhouse control + .3 percent CO <sub>2</sub> —7 days (age 31)	14.43	5.35	2.7
Greenhouse control + .3 percent CO <sub>2</sub> —15 days (age 39)	12.88	5.58	2.3
Greenhouse control + .3 percent CO <sub>2</sub> —20 days (age 44)	11.43	4.88	2.4
Greenhouse control grown in sand 46 days. 1926	21.23	2.77	7.7
Greenhouse control grown in soil 46 days. 1926	13.93	4.87	2.9

F. With flower stalks.

F.S.D. The same series flowered later when transferred to a short day.

(1). These plants flowered in a house adjacent to gantry crane house due to the entrance of diffused light of about 5 f.c.

(2). Some of these plants were sampled after 15 days and 3 out of 12 of this series flowered when returned to a short day. When sampled after 44 days flower stalks were appearing.

(3). None of the plants of this series flowered when returned to a short day.

(4). Sampled Feb. 28, 1927.

Note: Greenhouse 2, 1927 received daylight plus 12 hours of artificial light each night from the gantry crane. Carbon dioxide concentration about .3 percent. Temperature 78° F. 7 to 24 hour day plants grown in constant-light room with CO<sub>2</sub> concentration and temperature same as greenhouse 2. Greenhouses 1 and 2 in 1926 received 6 hours of light each night from the gantry crane. CO<sub>2</sub> concentration increased in 2. Temperature 68° F.

TABLE 44. Analysis of Radish Tops, Showing the Relation of Carbohydrate and Nitrogen Fractions to Length of Day. Constant-light Room

Day Length	Wet Weight per Plant	Percentage Moisture	Nitrogen Percentage				Acid Hy- drolyzable Percentage	Sucrose Percentage		Dextrose Percentage	Total Carbo- hydrates Percentage		
			Insoluble		Soluble			Total	Dry Weight		Dry Weight	Wet	Dry
			Dry Weight	Dry Weight	Dry Weight	Dry Weight							
1925													
7-hour	10	92.9	2.87	2.23	.36	5.11	7.58	.00	+	.54	7.58		
12-hour	20	92.7	2.21	1.79	.29	4.00	8.65	.00	4.37	.95	13.02		
17-hour	81	90.7	1.35	.46	.17	1.81	12.68	+	9.01	2.02	21.69		
19-hour	30	90.7	1.88	1.50	.31	3.38	12.56	+	9.41	2.05	21.97		
24-hour	16	84.9	1.62	1.05	.40	2.67	14.16	+	6.85	3.18	21.01		
1926													
5-hour	9.4	94.1	3.32	2.64	.36	5.96	8.56	.00	1.83	.62	10.39		
7-hour	8.7	93.7	2.86	2.20	.35	5.06	7.89	.69	3.71	.86	12.29		
12-hour	23.0	92.1	2.42	.82	.26	3.24	9.28	.00	4.14	1.07	14.42		
17-hour	33.0	90.6	2.06	.76	.26	2.82	12.97	.47	8.78	2.08	22.22		
19-hour	32.0	86.9	1.38	.34	.23	1.72	19.00	1.96	8.29	3.82	29.25		
24-hour	16.2	88.7	2.07	1.09	.36	3.16	13.15	1.11	7.23	2.44	21.49		
1927													
7-hour	7.0	92.1	2.75	3.58	.50	6.33	7.48	.00	1.47	0.71	8.95		
9-hour	13.0	92.7	2.49	2.21	.34	4.70	8.53	.00	2.82	0.84	11.35		
12-hour	17.0	93.9	2.44	2.79	.32	5.23	8.30	.00	.59	.55	8.89		
15-hour	28.0	91.8	2.03	1.33	.28	3.36	11.46	.00	3.95	1.26	15.41		
17-hour	20.0	86.7	1.42	1.03	.33	2.45	13.52	+	2.57	2.53	21.90		
19-hour	5.2	88.4	1.87	1.00	.34	2.87	11.26	1.85	6.53	1.57	17.15		
24-hour	8.0	90.8	2.28	1.72	.37	4.00		1.28	4.61				

+ Only a trace present.

TABLE 5. *Carbohydrate-nitrogen Relation in Lettuce, a Long-day Plant, Grown With Various Lengths of Day. Analyses of Whole Aërial Portion*

Treatment of Plant, and Number of Days in Growth Condition	Total Carbo- hydrate, % Dry Weight	Total Ni- trogen, % Dry Weight	Carbohydrate Nitrogen
1. Plants flowering or with flowering response			
Greenhouse 1—25 days—1925+ . . . . .	19.93	4.21	4.7
Greenhouse 2—25 days—1925+ . . . . .	27.35	4.58	6.0
17 hour day, 25 days—1925+ . . . . .	22.01	3.86	5.7
19 " " 25 " —1925+ . . . . .	24.08	4.06	6.0
24 " " 25 " —1925+ . . . . .	26.32	3.78	7.0
Greenhouse 1—60 days—1926+ grown in sand . . . . .	16.10	3.07	5.3
Greenhouse 1, 60 days—1926. Soil+ . . . . .	19.88	4.48	4.4
Greenhouse 2, 60 days—1926. Sand+ . . . . .	15.26	1.69	9.2
Greenhouse 2, 60 days—1926. Soil+ . . . . .	17.10	2.50	6.8
17 hour day, 60 days—1926+ . . . . .	19.44	2.88	6.8
19 hour day, 60 days—1926+ . . . . .	26.92	3.61	7.4
24 hour day, 60 days—1926+ . . . . .	28.54	3.12	9.2
15 hour day, 21 days—1927- . . . . .	23.10	5.56	4.2
15 hour day, 61 days—1927+ . . . . .	21.22	3.24	6.5
17 hour day, 20 days—1927- . . . . .	23.75	4.50	5.3
17 hour day, 61 days—1927+ . . . . .	20.84	2.37	8.9
19 hour day, 21 days—1927- . . . . .	26.68	4.68	5.7
19 hour day, 61 days—1927+ . . . . .	18.12	2.39	7.6
24 hour day, 19 days—1927- . . . . .	25.75	4.62	5.6
24 hour day, 45 days—1927+ . . . . .	38.28	4.85	7.9
2. Plants not flowering and with no flowering response			
Control greenhouse, 60 days—1925 . . . . .	18.57	4.53	4.1
5 hour day, 60 days—1925 . . . . .	13.53	4.73	2.9
7 hour day, 60 days—1925 . . . . .	20.92	4.04	5.2
12 hour day, 60 days—1925 . . . . .	21.04	4.59	4.6
Control greenhouse, 60 days—1926 . . . . .	22.11	4.75	4.7
7 hour day, 60 days—1926 . . . . .	18.30	4.89	3.7
12 hour day, 60 days—1926 . . . . .	17.46	4.14	4.2
Control greenhouse, 34 days—1927 . . . . .	22.86	4.95	4.6
5 hour day, 59 days—1927 . . . . .	14.86	4.11	3.6
9 hour day, 61 days—1927 . . . . .	18.05	5.09	3.6
12 hour day, 20 days—1927 . . . . .	12.08	2.73	4.5
12 hour day, 61 days—1927 . . . . .	18.80	4.21	4.5

+ Flower stalk visible.

- No flower stalk visible, would flower later.

5-24 hour day grown in constant-light room with artificial light entirely and with glass-water filter. 1925 series temperature 78° F. and illumination about 970 f.c. 1926 series temperature 68° F. and illumination about 1200 f.c. 1927 series 78° F. and 1200 f.c. illumination.

There is apparently no relation between percentage composition of carbohydrate and nitrogen and flowering in the radish, since these can be varied quite independently of the flowering. Flowering can be initiated by illuminating the radish for eight hours each night with 170 foot candles with no resultant accumulation of carbohydrates. Flowering can also be initiated with a much higher intensity, 700 foot candles (Greenhouse 1,

1926), for six hours each night, with considerable accumulation of carbohydrates. When the plants were grown in sand (Greenhouse 2, 1926) there was a further accumulation in total carbohydrates to the maximum for the series at 34.73 percent. Total nitrogen was reduced to 1.52 percent. These plants flowered. Control plants grown in sand on short days did not flower but accumulated considerable carbohydrate, 21.23 percent. Total nitrogen fell to 2.77 percent in this case. Of the plants grown in the constant-light room in 1927 on 7, 12, 15, 17, 19, and 24 hour days, the plants on the four longest day lengths flowered, while those of 12 hours and less did not flower. The effect of age of the plant at the time of sampling is shown in table 6. This is also discussed later. All radish plants listed in table 4 were grown from seed which was planted in each condition, except the 12 and 24 hour day, 12 and 24 hour night plants, and those grown with daylight plus eight hours of artificial light at 170 foot candles. These plants were grown in the control greenhouse and then transferred to the various conditions. It will be observed that the 24 hour day, 24 hour night and 12 hour day, 12 hour night plants in table 2 had practically the same percentage composition of carbohydrate and nitrogen. This might be expected since these plants were grown under the same conditions except for the duration of exposure to light. The set grown on the long day flowered while those on the 12 hour day did not flower. The soluble nitrogen in percentage of dry weight was 2.44 for the 12 hour and 2.82 for the 24 hour day plants as compared with 2.78 and 2.80 for the respective insoluble fractions. There is therefore no significant difference in these fractions. Soluble nitrogen in general parallels closely the total nitrogen values. The listing of this fraction has been omitted from table 4 and several other tables to save space.

It is of especial interest to note that the highest ratio of carbohydrate to nitrogen in radish is produced by an 18 hour day (Houses 1 and 2) where nitrate supply has been limited by growing the plants in sand. In this case large amounts of carbohydrate are produced, nitrogen supply is a limiting factor to further growth, and carbohydrates accumulate. This makes no difference with flowering since the plants flowered on the long day regardless of carbohydrate-nitrogen relations. When grown in sand in the control greenhouse on the normal day length the carbohydrate-nitrogen ratio of radish plants was also very high (7.7). The value was about equal to the ratio obtained (7.6) by growing plants in soil on a 19 hour day. The control plants did not flower while those grown on the 19 hour day flowered. The values given in table 4 are averages of a large number of plants. Any number can be grown similarly which will give individuals with values in very close agreement. Flowering in radish is therefore believed to be quite independent of the carbohydrate-nitrogen relations and depends only upon day length.

In table 4.4 are presented chemical data showing the trend of various

carbohydrate and nitrogen fractions in radish plants as day length increases. The long day plants represented in this table were flowering when sampled. The table shows the percentage composition of insoluble and soluble nitrogen and acid hydrolyzable, sucrose and dextrose, carbohydrate fractions on a dry weight basis. Percentage total nitrogen and total carbohydrate are given on both the green weight (wet) and dry weight basis. Weight per plant and percentage moisture are also given. As pointed out above total carbohydrates and weight per plant increase with day length to 17 or 19 hours while a corresponding increase is not maintained up to a 24 hour day. Weight per plant follows a similar curve. Total, soluble, and insoluble nitrogen on a dry weight basis decrease with increasing carbohydrates. The sucrose fraction is near zero on short day lengths but increases on 17, 19, and 24 hour days. This fraction decreases with age of plants. Only one other plant studied in these experiments contained as little sucrose. This was the coleus. Both the Golden Bedder and variegated varieties of this species gave no test for sucrose when grown on either long or short days.

The carbohydrate-nitrogen relation for lettuce grown under the different conditions is summarized in table 5. The analyses of plants listed in this table shows a considerable range of carbohydrate and nitrogen percentages both in the case of the plants which flowered or had the flowering response and in the plants which did not flower and had no flowering response. The data in this table are based on the analysis of only two to ten plants in each sample and are therefore much more variable than the data for radish presented in table 3 in which large numbers of individuals were used in each sample. There is no relation between the percentage carbohydrate or nitrogen and the tendency to flower in lettuce shown by these data. Carbohydrates, in general, increase with increasing length of day in both radish and lettuce. In contrast with radish, lettuce plants did not show any increase in carbohydrate when grown in sand as compared to a good soil.

In order to determine some of the effects of age of the plants at sampling time on carbohydrate and nitrogen fractions, a series of experiments was made in 1927. Lettuce and radish plants were analyzed first when the plants were young and vigorously vegetative and later when they were flowering or beginning to flower on longer day lengths. The data from this series of analyses are given in table 6. The weight per plant of lettuce increased with day length up to a 17 hour day while there was no proportional increase on 19 and 24 hour days. The aerial portion and roots of radish follow a similar curve. The amount of water in the aerial portion in general decreases with age. The acid hydrolyzable fraction increases slightly in lettuce with age. In the aerial portion of radish there is a slight decrease, while the roots show a tendency to increase. Sucrose, dextrose and total carbohydrates in general decrease with age in both



TABLE 6. *Some Effects of Age on the Chemical Composition of Radish and Lettuce Plants. 1927 Series. Whole Aerial Portion*

No. of Days in Condition, and Day Length	Wt. per Plant, Grams	% Moisture	Total Nitrogen %		Acid Hydro- lyzable %		Sucrose %		Dextrose %		Total Carbohy- drates %			
			Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry		
Lettuce, whole aerial portion														
Control greenhouse, 34 days.....	6.3	91.5	.42	4.95	.71	8.3	.81	9.5	.43	5.1	1.95	22.4		
Control greenhouse, 51 days.....	10.8	92.8	.33	4.64	.50	7.0	.42	5.9	.37	5.1	1.29	18.0		
12 hour day, 20 days.....	6.0	91.8	.23	2.73	.46	5.5	.28	3.4	.26	3.2	1.00	12.1		
12 hour day, 61 days.....	45.0	90.4	.24	2.52	.44	4.6	.35	3.7	.29	3.1	1.08	11.3		
15 hour day, 21 days.....	13.0	93.7	.35	5.56	.42	6.8	.57	9.1	.45	7.2	1.45	23.1		
15 hour day, 61 days.....	64.0	90.4	.31	3.24	.91	9.4	.73	7.6	.40	4.2	2.04	21.2		
17 hour day, 20 days.....	20.0	92.8	.33	4.50	.49	6.8	.71	9.9	.51	7.1	1.71	23.8		
17 hour day, 61 days.....	135.0	88.8	.27	2.37	1.23	10.9	.80	7.1	.32	2.8	2.35	20.8		
19 hour day, 21 days.....	29.0	93.1	.32	4.68	.56	8.1	.74	10.7	.54	7.8	1.84	26.7		
19 hour day, 61 days.....	53.0	86.6	.32	2.39	1.36	10.2	.70	5.2	.37	2.8	2.43	18.1		
24 hour day, 19 days.....	23.0	91.9	.37	4.62	.78	9.6	.78	9.6	.53	6.5	2.09	25.8		
24 hour day, 45 days.....	80.0	91.7	.40	4.85	1.31	15.8	1.18	14.3	.68	8.1	3.17	38.3		

TABLE 6.—Continued

No. of Days in Condition, and Day Length	Wt. per Plant, Grams	% Moisture	Total Nitrogen %		Acid Hydro- lyzable %		Sucrose %		Dextrose %		Total Carbohy- drates %		
			Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry	
Radish, whole aerial portion													
12 hour day, 20 days.....	2.2	92.0	.46	5.70	1.04	13.0	.23	2.9	.27	3.3	1.54	19.1	
12 hour day, 53 days.....	17.0	91.0	.32	3.49	1.02	11.0	+	+	.40	4.3	1.47	15.9	
15 hour day, 20 days.....	3.0	92.2	.37	4.69	1.00	12.8	.25	3.2	.13	1.7	1.38	17.7	
15 hour day, 52 days.....	28.0	91.8	.28	3.36	.94	11.5	+	+	.32	4.0	1.26	15.4	
17 hour day, 20 days.....	4.3	91.6	.41	4.84	1.25	14.9	.25	3.0	.27	3.2	1.77	21.1	
17 hour day, 45 days.....	20.0	86.7	.33	2.45	.90	6.7	+	+	.34	2.6	1.28	9.6	
19 hour day, 20 days.....	4.4	90.9	.42	4.67	1.51	16.6	.13	1.47	.35	3.8	1.99	21.9	
19 hour day, 40 days.....	5.2	88.4	.34	2.87	1.56	13.5	.21	1.85	.76	6.5	2.53	21.9	
24 hour day, 19 days.....	3.7	89.1	.51	4.68	1.97	18.1	.32	3.00	.47	4.4	2.76	25.5	
24 hour day, 35 days.....	8.0	90.8	.37	4.00	1.03	11.3	.12	1.28	.42	4.6	1.57	17.2	
Radish, roots only													
12 hour day, 20 days.....	1.0	92.2	.25	3.20	.68	8.7	.20	2.57	1.88	24.1	2.76	35.4	
12 hour day, 53 days.....	23.0	93.0	.18	2.60	.65	9.3	.19	2.72	1.72	24.6	2.56	36.7	
15 hour day, 20 days.....	1.4	93.1	.20	2.91	.62	9.0	.10	1.46	1.79	26.1	2.51	36.5	
15 hour day, 52 days.....	17.0	93.5	.14	2.16	.78	12.0	.06	.93	.59	9.1	1.43	22.1	
17 hour day, 20 days.....	4.1	93.6	.19	2.96	.51	8.0	.15	2.34	2.06	32.1	2.72	42.4	
17 hour day, 45 days.....	6.4	93.8	.16	2.59	.57	9.2	.28	4.54	.18	2.9	1.03	16.7	
19 hour day, 20 days.....	3.0	92.7	.21	3.10	.68	9.3	.25	3.41	2.37	32.3	3.30	45.0	
19 hour day, 40 days.....	12.0	90.5	.19	2.00	1.49	15.7	.25	2.64	.91	9.6	2.65	27.9	
24 hour day, 19 days.....	1.2	89.3	.33	3.07	1.10	10.3	.42	3.91	2.90	27.0	4.42	41.2	
24 hour day, 35 days.....	5.9	89.7	.28	3.79	1.49	14.5	.24	2.33	1.20	11.7	2.93	32.5	

lettuce and radish. It is therefore important for purposes of comparison to choose plants of these two species which are approximately the same age.

The analytical data for salvia, a short day plant, are presented in table 7. This plant flowers well up to and including day length of 15 hours, rarely on a 17 hour day. A photograph showing the flowering on short day lengths is included as text figure 6. The carbohydrate and nitrogen fractions show a very narrow range over all day lengths from five hours to 24. On the shortest day length, five hours, in 1926, carbohydrates were high at 22.87 percent as compared with 28.19 percent on the 19 hour day, while nitrogen showed a correspondingly small variation from 3.24 percent to 2.04 percent on the same range of day lengths. The application of large amounts of nitrate to the soil made practically no difference in the total nitrogen content, although the plants showed considerable foliar injury due to the toxicity of high concentrations of this salt. The seven hour day plant receiving extra nitrate had a total nitrogen percentage of 3.35 as compared to the value 2.8 for the seven hour with no extra nitrate. The carbohydrate percentages were 14.08 and 25.45, respectively. The slight increase in nitrogen may as well be attributed to the falling off of carbohydrate due to slight foliar injury as to the increase in nitrate supply in the soil. The 17 and 19 hour day plants receiving nitrate were also slightly higher in total nitrogen and slightly lower in total carbohydrate, but compared with plants growing on short day lengths total nitrogen in those plants receiving large quantities of nitrate is very low. Salvia is able to regulate closely the total percentage composition of nitrogen in the tissues when grown in a medium with high nitrogen supply. The numerical value of this percentage depends mainly upon length of day and intensity of light. This regulatory action apparently is not restricted to salvia since various workers have found a similar relation in other species. Walster (20) found that when barley was grown at a high temperature with a high nitrogen supply in a sand medium the plants were very weak and prostrate in growth habit, whereas when grown similarly except in a cool house the plants were erect and sturdy. He found no greater differences in total nitrogen percentages, however, than may be easily attributed to variations in sampling. Hooker and Bradford (7, 8) have analyzed both the bearing fruit spurs and bark of apple twigs which have been fertilized with nitrate, ammonium sulfate, and blood. As compared with the control plants with no fertilizer they found no appreciable differences in total nitrogen percentage. The interesting thing about these observations is that the effects of high nitrogen supply are brought about in these species with no corresponding increase in percentage total nitrogen in the plants. Woo (22) found that high nitrate content of soil produced no corresponding increase of nitrogen in amaranthus plants. Tincker (19) found that the crude protein ( $N \times 6.25$ ) in the leaves of cocksfoot grass plants could be about doubled when the plants were fed sodium nitrate at the rate of five

TABLE 7. *Carbohydrate-nitrogen Relation in Salvia, a Short-day Plant. Analyses of Whole Aërial Portion Unless Otherwise Stated*

Treatment of Plant, Age and Number of Days in Growth Condition	Total Carbo- hydrate, % Dry Weight	Total Ni- trogen, % Dry Weight	Carbohydrate Nitrogen
1. Plants flowering or with flowering response			
Control greenhouse, 62 days—1926.....	18.47	3.40	5.5
5 hour day, 62 days—1926.....	22.87	3.24	7.1
7 hour day, 62 days—1926.....	25.45	2.80	9.1
12 hour day, 62 days—1926.....	27.63	3.16	8.8
7 hour day, NaNO <sub>3</sub> , 62 days, 1926 (1).....	14.08	3.35	4.2
12 hour day, 35 days—1927 (2).....	31.62	3.84	8.2
17 hour day, 35 days—1927 (2).....	33.56	3.52	9.6
5 hour day, 61 days—1927.....	23.88	4.39	5.5
7 hour day, 61 days—1927.....	23.86	3.99	6.0
9 hour day, 61 days—1927.....	25.39	3.71	6.8
12 hour day, 61 days—1927.....	30.59	2.49	12.3
15 hour day, 61 days—1927.....	30.45	2.47	12.3
17 hour day, 61 days—1927.....	30.74	2.84	10.8
2. Plants not flowering and with no flowering response			
Greenhouse 1—62 days—1926.....	14.48	3.51	4.1
Greenhouse 2—62 days—1926.....	20.82	2.70	7.6
17 hour day, 62 days—1926.....	25.41	1.93	13.2
19 hour day, 62 days—1926.....	28.19	2.03	13.8
24 hour day, 62 days—1926.....	26.18	2.26	11.6
17 hour day, NaNO <sub>3</sub> , 62 days—1926 (1).....	23.62	2.34	10.1
19 hour day, NaNO <sub>3</sub> , 62 days—1926 (1).....	17.64	2.80	6.3
24 hour day, 35 days—1927.....	37.95	3.03	12.4
19 hour day, 61 days—1927.....	31.50	2.56	12.3
24 hour day, 61 days—1927.....	35.14	3.08	11.4
Greenhouse 2—61 days—1927.....	25.33	2.97	8.5
Greenhouse + 6 hours light each night, Jan. 28—Oct. 4, 1927. Leaves only (3).....	22.12	2.66	8.4
Same except stems only (3).....	21.27	.57	37.3
Same except from Jan. 28—Dec. 19, 1927. Leaves only (3).....	22.18	2.53	8.4
Greenhouse control sampled Oct. 4, 1927. Leaves only.....	16.81	3.31	5.1
Same except stems only.....	21.66	.79	27.3

(1). Given 100 cc. of NaNO<sub>3</sub> solution containing 5 grams NaNO<sub>3</sub> at one or two week intervals in four separate doses.

(2). Other 12 and 17 hour day plants of this series were flowering when sampled after 62 days. The 17 hour day plants flowered only at the terminal while the 12 hour day plants flowered at both terminals and laterals.

(3). Received 6 hours artificial light each night from one 1000-watt lamp.

Note: 5 to 24 hour day plants grown in constant-light room. In 1926 the temperature was 68° F. and illumination about 1200 f.c. In 1927 the temperature was 78° F. and illumination about 1200 f.c. Greenhouses 1 and 2 in 1926 received 6 hours of light each night from the gantry crane. CO<sub>2</sub> concentration was increased in 2. Temperature 68° F.

grams weekly. This was true of plants grown either on a ten hour day or on the normal day length of June. It is evident therefore that not all plants are capable of a close regulation of total nitrogen percentage. Nightingale (16) has compared the analysis of stems from salvia plants grown on a short day of seven hours in the greenhouse during February, March, and April with those similarly grown except with full daylight supplemented at night by six hours of low intensity artificial light. The total carbohydrate in the lower stems on a percentage dry weight basis was 24.4 for the long-day plants and 23.9 for the short-day plants. The upper stems contained 19.3 and 20.0, respectively. Total nitrogen percentage of dry weight was .7 to 1.1 for the lower stems as compared with 2.0 to 1.6 for the upper from the long and short day plants. The total nitrogen percentage for the whole plant was 2.7 to 2.7. There is, therefore, no significant differences in carbohydrate or total nitrogen percentages between the long and short-day type in plants reported by Nightingale. This is probably due to the fact that the long-day plants received additional light of such a low energy value that very little additional photosynthesis took place as compared with the seven-hour day series, while the day length effects in initiating flowering are produced at a very low intensity. Nightingale found that all forms of soluble nitrogen except nitrate were relatively low in short-day salvia plants. He concludes from this that salvia is limited by a seven-hour day in the assimilation of nitrate. Since one set of plants is flowering and the other is not it would be as reasonable to conclude that other forms of soluble nitrogen were being used up by the flowers in case of the short day plants. Consequently these forms should be lower in amount in the stems. The point of especial interest is that total nitrogen and carbohydrate remain practically the same regardless of flowering.

Returning again to table 7 it will be noted that even with the narrow range of carbohydrate there is a definite increase with day length. The ability to flower is not associated with a decrease in carbohydrates since plants grown in Greenhouse 1 in 1926 with six hours of supplementary light did not flower with carbohydrate at a low value of 14.48 percent. In the 1927 series plants in one of the control greenhouses near the gantry crane house did not flower on account of diffuse light reaching these pots each night. The illumination value was less than ten foot candles. The analyses of these plants appear in table 8. The total carbohydrate percentage was 20.23. Similar plants in the control house farthest away from the crane received a much lower intensity of diffused light and did not flower. Analyses of these plants are given in table 8. The total carbohydrate percentage was 17.93. The analyses of salvia plants grown in the greenhouse with six hours of additional light each night from January until October and December are of interest. These plants were prevented from flowering during this entire period, while control plants flowered in the greenhouse in March, April, September and October. The carbohydrate

TABLE 8. *Some Effects of Day Length on the Chemical Composition of Salvia Plants. Analysis of Whole Aerial Portion*

Conditions of Growth	Wt. per Plant, Grams	% Moisture	Nitrogen %			Carbohydrate %							
			Soluble		Total	Acid Hydrolyzable		Sucrose		Dextrose		Total	
			Dry	Wet		Dry	Wet	Dry	Wet	Dry	Wet		
					Dry							Wet	Dry
1926 Series. 62 days in conditions													
Control greenhouse*	135	86.1	.74	.47	3.40	2.01	14.41	.29	2.10	.27	1.96	2.57	18.47
Greenhouse 1	70	85.7	.68	.51	3.51	1.68	11.72	.24	1.70	.15	1.06	2.07	11.48
Greenhouse 2	185	85.7	.48	.39	2.70	2.39	16.70	.32	2.32	.21	1.50	2.93	20.52
5 hour day*	93	87.7	.80	.40	3.24	2.49	20.24	.12	.97	.20	1.66	2.81	22.87
7 hour day*	126	84.6	.79	.43	2.80	3.43	22.24	.27	1.76	.22	1.45	3.92	25.45
12 hour day*	151	81.3	.47	.59	3.16	4.51	24.12	.38	1.96	.29	1.55	5.18	27.63
17 hour day*	18	92.5	.46	.34	1.93	4.15	23.65	.12	.68	.19	1.08	4.46	25.41
19 hour day*	35	80.7	.40	.40	2.03	5.11	26.41	.17	.88	.17	.90	5.45	28.19
24 hour day	20	79.2	.44	.47	2.26	4.79	23.04	.38	1.86	.27	1.28	5.44	26.18
1927 Series. 61 days in conditions													
(1) Control greenhouse	78	85.2	.90	.57	3.87	2.34	15.77	.50	3.39	.16	1.07	3.00	20.23
Control greenhouse*	100	87.0	1.18	.54	4.20	1.78	13.67	.29	2.23	.26	2.03	2.33	17.93
Greenhouse 2	341	87.0	.87	.38	2.97	2.78	21.29	.36	2.80	.16	1.24	3.30	25.33
5 hour day	43	89.6	1.54	.46	4.39	2.03	19.61	.33	3.21	.11	1.03	2.47	23.85
7 hour day*	132	89.4	1.47	.43	3.99	2.23	20.89	.22	2.02	.10	.95	2.55	23.86
9 hour day*	129	89.1	1.25	.41	3.71	2.44	22.31	.23	2.06	.11	1.02	2.78	25.39
12 hour day*	156	86.8	.83	.33	2.49	3.53	26.69	.38	2.88	.13	1.02	4.04	30.59
15 hour day*	151	84.2	.62	.39	2.47	3.89	24.68	.57	3.61	.34	2.16	4.80	30.45
(2) 17 hour day*	244	87.7	.81	.35	2.89	3.28	26.60	.33	2.61	.19	1.53	3.80	30.74
19 hour day	346	86.2	.71	.36	2.56	3.80	27.53	.36	2.59	.19	1.38	4.35	31.50
24 hour day	179	84.1	.94	.43	3.08	4.22	30.58	.45	3.23	.18	1.33	4.85	35.14

\* Flowering.

(1). Did not flower on account of diffused light of less than 10 foot candles from gantry crane greenhouse.

(2). Flowered on one terminal only. Greenhouses 1 and 2 in 1926 received 6 hours extra light each night from gantry crane. No. 2 also received extra CO<sub>2</sub>. Temperature 68° F. In 1927 2 received 12 hours each night from the crane making a 24 hour day, 12 hours of which was daylight. Temperature 78° F. All other day-length plants were grown in the constant-light room.

and nitrogen fractions showed very little change at the end of this exposure, as is indicated in table 7.

The variation of various fractions of carbohydrate and nitrogen constituents of salvia plants on different day lengths are shown in table 8. The 1926 series was grown at a temperature of 68° F. as compared with 78° F. in the 1927 series. Weight per plant is maximum on a 12 hour day in the low temperature series as compared with a 19 hour day in the high temperature series. Salvia grows better at the higher temperature. The data show an increase in the weight per plant, total carbohydrates, and nitrogen in those grown at the higher temperatures as compared with the low temperature series. The increase in nitrogen appears in both the soluble and total fractions. The increase in carbohydrate is mainly in the acid hydrolyzable and sucrose fractions. Total carbohydrate increases with day length and nitrogen decreases in both the 1926 and 1927 series. An especially noteworthy characteristic of this plant is the ability to maintain a high total carbohydrate value on a five and seven hour day, resulting in a comparatively narrow range in percentage carbohydrate between a five and a 19 hour day.

The data from the analyses of buckwheat plants grown on various day lengths are especially interesting (table 9). This plant flowers on all day lengths from five to 24 hours (fig. 7). The height varies from about 18 inches on a five hour day to 52 inches on a 19 hour day. Total carbohydrates increase and total nitrogen decreases from a five to a 24 hour day. Total nitrogen usually decreases to less than one percent of the dry weight on a 24 hour day. The weight per plant increases regularly with day length up to a 19 hour day. A corresponding increase is not maintained up to continuous 24 hour illumination. The leaves of the 24 hour day plants show considerable injury as compared with the 17 hour day plants, but buckwheat in contrast to tomato, is able to continue to grow and flower on a 24 hour day. The injury from continuous light consists in the slight dying back of the leaf margins for a short distance and as the inner region of the lamina continues to grow the margin has a tendency to turn upward producing a shallow cuplike appearance. This effect can be seen in text figure 7 on both the 19 and 24 hour day plants.

Data have already been presented showing that the percentage composition of total nitrogen and total carbohydrate has little effect on flowering in the species of long and short day plants studied or in the everblooming types. In general, the percentage of carbohydrates increases with length of day where light intensity is high accompanied by a decrease in nitrogen. Flowering is initiated by a long or short day depending upon the species or variety, and is independent of the percentage composition of total easily available carbohydrates. In other species, like buckwheat, flowering is not affected by either day length or carbohydrate composition. Garner and Allard (5) have shown that day length effects are localized in each

branch of the plant. Knott (11) has shown further that the effect in cosmos is probably restricted to a few cells at the growing point of each branch. The effect of light in initiating flowering may be directly upon the protoplasm of the cells at the growing point with no resulting change

TABLE 9. *Everblooming Plant. Carbohydrate-nitrogen Relation in Buckwheat which Flowered on All Lengths of Day. Whole Aërial Portion*

Treatment of Plant and Number of Days in Growth Conditions	Total Carbohy- drate, % Dry Weight	Total Ni- trogen, % Dry Weight	Carbohydrate Nitrogen	Weight per Plant, Grams
(1) 12 hour day, 23 days—1924 . . . .	17.56	2.61	6.7	11.5
(1) 17 hour day, 23 days—1924 . . . .	29.90	1.93	15.4	33.6
(1) 19 hour day, 23 days—1924 . . . .	28.62	2.08	14.0	29.0
(1) 24 hour day, 23 days—1924 . . . .	35.66	1.68	21.2	27.4
(1) 17 hour day, 65 days—1924 . . . .	28.49	.80	35.5	143.0
(1) 24 hour day, 65 days—1924 . . . .	28.49	.46	62.0	104.0
Control greenhouse, 40 days—1925 . .	17.99	3.72	4.8	16.1
(4) Greenhouse 1, 33 days—1925 . . .	17.10	3.16	5.5	47.3
(5) Greenhouse 2, 33 days—1925 . . .	32.36	.98	33.0	78.8
(2) 5 hour day, 69 days—1925 . . . .	15.84	3.44	4.6	3.5
(2) 12 hour day, 33 days—1925 . . . .	19.80	3.17	6.2	20.9
(2) 17 hour day, 33 days—1925 . . . .	38.35	1.42	27.0	42.6
(2) 19 hour day, 33 days—1925 . . . .	36.29	1.11	32.5	56.8
(2) 24 hour day, 33 days—1925 . . . .	39.41	1.16	33.8	—
Control greenhouse, 58 days—1926 . .	23.15	3.05	7.7	11.5
(4) Greenhouse 1, 58 days—1926 . . .	27.62	2.25	12.3	32.7
(5) Greenhouse 2, 58 days—1926 . . .	37.19	1.08	34.5	47.2
(3) 5 hour day, 58 days—1926 . . . .	25.44	3.39	7.5	3.2
(3) 7 hour day, 58 days—1926 . . . .	32.93	2.74	12.0	4.7
(3) 12 hour day, 58 days—1926 . . . .	32.10	2.11	15.2	12.5
(3) 17 hour day, 58 days—1926 . . . .	31.15	1.01	31.0	31.6
(3) 19 hour day, 58 days—1926 . . . .	33.26	1.15	28.8	48.5
(3) 24 hour day, 58 days—1926 . . . .	36.93	.98	36.6	24.5
Control greenhouse, 64 days—1927 . .	7.94	3.16	2.5	7.1
(6) Greenhouse 2, 64 days—1927 . . .	35.57	.84	42.5	45.4

(1). Grown in constant-light room with artificial light entirely and with glass-water filter. Temperature 78° F. Average illumination about 450 f.c. on soil.

(2). Grown same as (1) except average illumination about 800 f.c. on soil.

(3). Grown same as (1) and (2) except temperature 68° F. and average illumination 1200 f.c.

(4). Greenhouse 1 daylight plus 6 hours from the gantry crane each night. Temperature in 1925 experiments 78° F. in 1926 68° F.

(5). Greenhouse 2 same as (4) except extra CO<sub>2</sub> concentration .3 percent.

(6). Greenhouse 2 in 1927 received 12 hours artificial light, otherwise same as (5).

in composition which may be detected by chemical analysis. Knott has shown that the change from the vegetative to flowering condition in spinach and cosmos is accompanied by a decrease in catalase at the terminals. Whether the decrease in catalase is a cause of flowering or is only associated with the beginning of flower production is still in doubt. It is believed,



however, that a study of enzymes or other substances present in very small amounts in the growing tips or elsewhere offers much more promise than gross carbohydrate and nitrogen fractions in various plant organs, in explaining the mechanism of light in initiating flowering and fruit production in the plant. Since some correlations have been found in a few species between tuberous root formation and the initiation of flowering, a search is in order for specific substances or stimuli accomplishing such correlation between root and tip of stem.

### Carbohydrate-Nitrogen Ratio in the Tomato

Starting with the work of Kraus and Kraybill (13) considerable study has been made of the carbohydrate-nitrogen relation in the tomato plant. These authors conclude that plants grown with an abundant supply of available nitrogen and the opportunity for carbohydrate synthesis are unfruitful, high in moisture, total nitrogen, and nitrate nitrogen, and low in reducing substances, sucrose, and polysaccharids. Fruitfulness, they found, was associated neither with highest nitrates nor highest carbohydrates, but with a condition of balance between them. Plants grown with a low nitrogen supply were found to be unfruitful, low in moisture and total nitrogen, and high in carbohydrates.

In the present study the same soil mixture was used in all studies with tomato plants, except where an attempt was made to induce recovery in tomatoes injured by exposures to continuous illumination. This will be discussed later. The soil mixture contained about one-fourth manure and the usual nitrate, potash, and phosphate salts which gardeners normally use to make up a good greenhouse soil. It is believed that the plants had all the nutrient salts which they could use during their growth period. All plants were grown in two-gallon glazed stoneware jars, except those on short tests of one to two weeks. This gave the roots ample space to produce full grown plants with many fruits.

The effect of various lengths of day on the carbohydrate-nitrogen fraction of tomato plants is shown in table 10. Carbohydrates increase with day lengths up to a 17 hour day, show no further increase on a 19 hour day, and a decrease on the 24 hour day. The 24 hour day plant had in each case become almost completely defoliated before these samples were taken. Total nitrogen decreases steadily with increasing day length up to a 17 hour day where it reaches a minimum of about one percent of the dry weight of the plant. The carbohydrate-nitrogen ratio increases with day length up to 17 or 19 hours. The plants set fruit on all day lengths from seven to 19 hours but did not fruit on either five or 24 hour day. In contrast to the observations of Kraus and Kraybill (13) it is seen that, under the above conditions, the ratio of carbohydrate to nitrogen has little to do with fruiting in the tomato. Fruiting is here taken to mean the setting and continued growth of three or four fruits per plant. High

TABLE 10. *Carbohydrate-nitrogen Relation in Tomato Plants Grown With Various Lengths of Day. Analyses of Whole Plants Except Roots and Fruits*

Treatment of Plant and Number of Days in Growth Conditions	Total Carbohydrate, % Dry Weight	Total Nitrogen, % Dry Weight	Carbohydrate/Nitrogen	Weight per Plant, Grams
Control greenhouse, 62 days—1924 F.	19.14	2.35	8.2	556
5 hour day, 62 days—1924 F.	14.88	3.30	4.5	142
7 hour day, 62 days—1924 F.	19.07	2.52	7.5	312
12 hour day, 62 days—1924 F.	25.43	2.22	11.4	528
17 hour day, 62 days—1924 F.	31.83	1.85	17.2	723
19 hour day, 62 days—1924 F.	33.84	1.77	19.1	634
24 hour day, 62 days—1924 F.	20.45	2.82	7.3	139
Control greenhouse, 40 days—1925 F.	20.53	2.46	8.3	645
5 hour day, 70 days—1925 F.	19.95	4.31	4.6	41
7 hour day, 70 days—1925 F.	14.10	3.45	4.1	444
12 hour day, 70 days—1925 F.	33.00	1.09	30.0	602
17 hour day, 70 days—1925 F.	34.94	1.16	30.0	569
19 hour day, 70 days—1925 F.	31.94	1.45	22.0	487
24 hour day, 70 days—1925 F.	1.24	3.74	—	11
Greenhouse 1, 70 days—1925 F.	25.75	1.34	19.2	780
Greenhouse 2, 70 days—1925 F.	34.02	1.35	25.1	1433
Greenhouse 2, 34 days—1926 F.	29.61	4.34	6.8	70
Greenhouse 1, 63 days—1926 F.	21.82	1.88	11.5	512
Greenhouse 2, 63 days—1926 F.	37.23	.96	39.0	471
12 hour day, 34 days—1926 F.	29.84	3.56	8.4	86
17 hour day, 34 days—1926 F.	37.17	2.29	16.3	149
19 hour day, 34 days—1926 F.	17.69	3.29	5.4	147
5 hour day, 63 days—1926 F.	9.84	5.29	1.9	20
7 hour day, 63 days—1926 F.	17.15	3.83	4.5	43
12 hour day, 63 days—1926 F.	25.45	1.27	20.0	261
17 hour day, 63 days—1926 F.	43.64	.96	45.5	278
19 hour day, 63 days—1926 F.	34.61	1.09	31.7	215
24 hour day, 63 days—1926 F.	11.33	4.35	2.6	29

## F. Fruiting.

5 to 24 hour day plants grown in constant-light room with artificial light entirely and with glass-water filter. 1924 series temperature 78° F. and illumination about 450 f.c. 1925 series same temperature but illumination about 800 f.c. 1926 series grown at 68° F. and illumination about 1200 f.c.

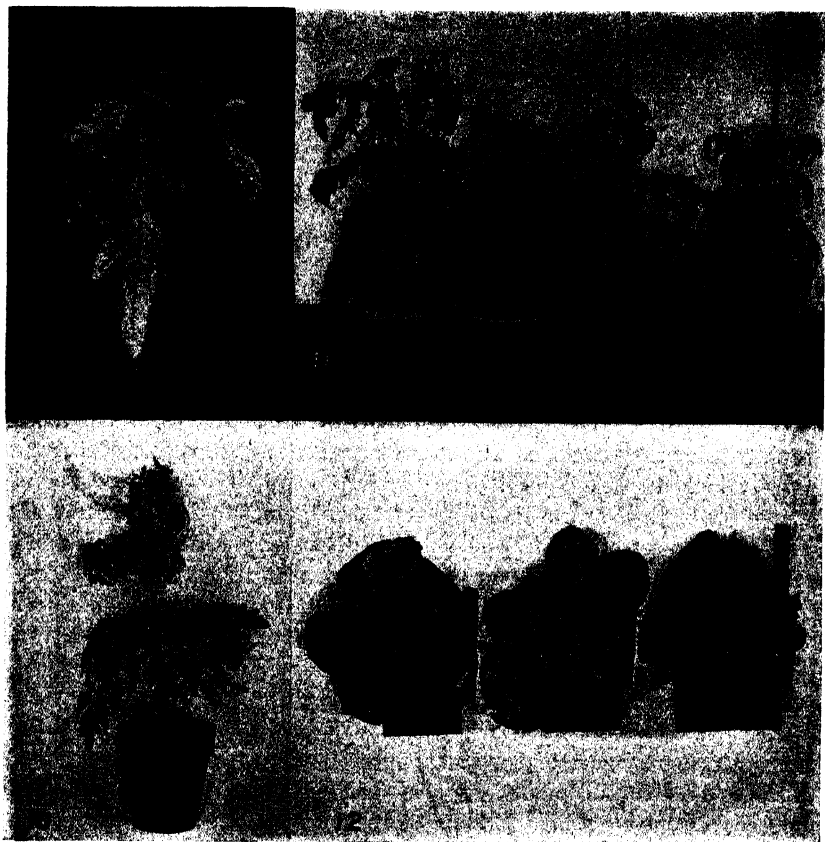
Greenhouses 1 and 2 received 6 hours illumination each night from the gantry crane. Temperature for respective years same as 5 to 24 hour day plants. Greenhouse 2 also received extra CO<sub>2</sub> at about .3 percent concentration.

carbohydrate accumulation results in a rapid depletion of nitrogen fractions in tomato. This agrees with the observations of Kraus and Kraybill. High carbohydrate accumulation does not, however, result in a condition of unfruitfulness where soil nitrogen is available. No study has been made in these experiments of the ability of the tomato plant to absorb nitrate nitrogen. It is possible that this plant may absorb sufficient nitrate to produce unfruitfulness under ordinary greenhouse conditions. It is also possible that when grown in sand with limited nitrate supply a condition

of unfruitfulness may be produced. This condition might also be produced by growing plants with a shortage of other mineral nutrients. The weight per plant increased with day length up to a 17 hour day in the case of those plants grown in the constant light room in the 1924 series. The weight of the 19 hour day plants decreased slightly. In the 1925 series with intensity almost twice as high the injury was greater on the long days of 17, 19 and 24 hours so that the greatest weight was produced on a 12 hour day. As the intensity increases the length of day for maximum growth decreases in case of the tomato plant. At the low intensity of 1924 even the 24 hour day plant produced considerable increase in weight before it had lost much of its leaf tissue. The weight per plant produced in the 1926 series is not comparable since this series was grown at a lower temperature (68° F.) which is not favorable to tomato. The weight of tissue produced at all day lengths at the low temperature is much lower than either the low intensity series of 1924 or the high intensity series of 1925. The 1926 series was also grown at a higher light intensity, 1200 foot candles. This operates to cause a decrease in weight produced at all of the longer day lengths. The amount of injury therefore depends both upon intensity and day length. The greatest weight produced during these experiments was that in Greenhouse 2 in 1925 of 1433 grams per plant in 70 days. These plants were grown with daylight supplemented by six hours each night from the crane with about ten times the normal carbon dioxid concentration. This combination of light produced very little if any leaf injury. Each plant produced from six to ten large fruits some of which were ripening when sampled. The ratio of total carbohydrate percentage to total nitrogen percentage was 25.1.

In the first experiments at low light intensity in 1924 it was evident that tomato plants would not withstand continuous illumination. After the plants had been in the condition 20 days only a few small leaflets at the terminals remained alive. Those plants grown on a 19 hour day in this series retained practically all of the leaves produced, all remained green and presented a normal appearance although they grew tall due to the low intensity. With higher intensities in 1925 and 1926 the 24 hour day plants showed the first signs of injury in about five days and after four weeks not a green leaf was left on any of the plants. The 19 hour day plants developed the injury more slowly and managed to maintain several green leaves all during the experiment but the older leaf tissue was injured severely. The 17 hour day plants at the higher intensity also appeared to be slightly injured, and from the data on weight per plant in table 10 it is evident that both the 17 and 19 hour day plants were injured.

Of the plants grown in these experiments the tomato is the most sensitive to high light intensity in combination with a long day. Many plants will withstand continuous illumination with little apparent injury, others are much more susceptible. These will be discussed later.



TEXT FIG. 9. A close-up photograph of a leaf from the tomato plant in FIG. 10, showing the necrotic areas developing along the veins. TEXT FIG. 10. A tomato plant kept under continuous illumination (24 hour day) in the constant-light room in 1927 for six days. The lower leaves are yellowing while the younger leaves around the terminal have a tendency to curl back toward the main stem. TEXT FIG. 11. Tomato plants grown in the constant-light room on 17 and 24 hour days, in the control greenhouse (House 1) and in the gantry crane greenhouse on a combination of daylight supplemented by 12 hours artificial light each night (House 2). Both the 24 hour day and House 2 plants have many yellowing leaves due to continuous illumination. In conditions 20 days. TEXT FIG. 12. Cabbage plants grown in the constant-light room on 5, 7, and 17 hour days in 1925 showing the buckling and splitting of leaves on the long days when grown under a fixed light source. The 5 hour day leaves tend to remain flat.

### **Further Experiments on the Injury to the Tomato Plant of Continuous Illumination**

Further experiments were made in 1926, 1927, and 1928 to determine a possible mechanism of injury of long days on the tomato plant. It was found that the first signs of injury appeared in five to seven days under continuous illumination. The leaves usually became faintly mottled with necrotic areas developing along the veins. Text figure 9 illustrates such a leaf from a plant under continuous illumination for six days. The leaves turn downward and backward toward the stem and slowly die back until after two or three weeks of exposure only a few small terminal leaves remain. Figure 10 shows the characteristic appearance of a tomato plant after six days of continuous illumination. The leaf shown in figure 9 was taken from this plant. New leaves appearing at the terminal become smaller and smaller until finally the whole plant dies if the intensity is kept sufficiently high. Since considerable amounts of carbohydrate accumulated on the long days of the 1924, 1925, and 1926 experiments it was at first thought that they accumulated in the leaves so much more rapidly than they could be translocated or used that this might account for the long day leaf injury. The chemical composition of whole plants grown for 63 days in continuous illumination in 1926 is given in table 11, part 1. In part 2 of this same table is given an analysis of stems and leaves grown at the same time but sampled after only seven days of continuous exposure, when the injury was just appearing. These plants were all young and vigorously vegetative. It will be observed that in part 1, percentage total carbohydrates increase with day length up to a 17 hour day and percentage nitrogen decreases in old plants that have been kept under artificial illumination for 63 days. In part 2 the leaves of 24 hour day plants are much higher in total carbohydrates and lower in total nitrogen than control plants grown in the greenhouse. Analyses of stems and petioles gave a similar increase in carbohydrate but less in magnitude. If the injury developed as a result of an accumulation of carbohydrates it should be feasible to choose a light intensity which would not permit of any accumulation and in this way protect the plants. Plants were therefore grown in the light room on different intensities, using two 1500-watt lamps without filters and placing the plants at various distances from the lamps. Analyses of plants so grown at various intensities are given in table 11, part 2. All of the plants in this table showed the typical long day injury except the controls grown in the greenhouse with daylight only. It will be seen that at an illumination of 150 foot candles carbohydrates do not accumulate in the leaves and that the total carbohydrate is less than in those grown in the control greenhouse during March, yet the injury develops, although more slowly. At 400 and 700 foot candles the injury develops at the usual rate so that after about three weeks' exposure the plant is almost completely defoliated. Total carbohydrates at 400 foot candles are at a

TABLE II. *Percentage Composition of Tomatoes as Affected by Day-length and Other Conditions*  
 I. After being kept under artificial light for 63 days. 1926. Entire plant except roots

Growth Conditions	Moisture %	Nitrogen				Carbohydrates, % Wet and Dry Weights							
		Soluble		Total		Acid Hydrolyzable		Sucrose		Dextrose		Total Carbohydrate	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
5 hour day.....	95.7	.14	3.2	.23	5.3	.43	9.8	+	+	+	+	.43	9.8
7 hour day.....	91.4	.14	1.6	.33	3.8	1.24	14.3	.11	1.2	.14	1.6	1.49	17.2
12 hour day.....	88.1	.11	.9	.27	1.3	2.44	20.4	.20	1.7	.40	3.3	3.04	25.5
17 hour day.....	86.8	.02	.2	.13	1.0	4.49	33.9	.40	3.0	.89	6.7	5.78	43.6
19 hour day.....	86.1	.02	.2	.15	1.1	4.01	28.9	.22	1.6	.58	4.2	4.81	34.6
24 hour day.....	91.8	.21	2.5	.36	4.4	.81	9.9	.04	.5	.08	1.0	.93	11.3

TABLE II.—*Continued*  
 2. Young tomato plants grown in greenhouses and then exposed to continuous illumination for 5 to 7 days.  
 All of these plants showed typical long day injury except controls grown in greenhouse  
 A. Analysis of leaves only

Growth Conditions and Sampling Date of Control Plants	Wt. per Plant, Grams	% Moisture	Nitrogen % Dry Weight	Carbohydrates, % Wet and Dry Weights							
				Acid Hydrolyzable		Sucrose		Dextrose		Total Carbohydrate	
				Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Control 3/28/1928	33	87.7	3.73	2.23	18.16	.24	1.95	.44	3.57	2.91	23.68
Control 4/16/1928	21	86.2	3.10	3.46	24.94	.12	.83	.44	3.16	4.02	28.93
Control 5/5/1928	15	86.3	3.10	3.28	23.89	.20	1.49	.27	2.00	3.75	27.38
Same, except sampled after 17 hours in dark room	16	86.7	3.58	2.32	17.45	.04	.26	.20	1.48	2.56	19.19
24 hour day, 700 f.c. (1)	15	85.7	2.78	4.00	27.95	.16	1.11	.32	2.25	4.48	31.31
Same, but sampled after 40 hours in dark room (1)	16	88.9	3.85	1.06	9.49	0	0	.13	1.20	1.19	10.69
24 hour day, 150 f.c. (1)	23	90.8	4.10	.74	8.00	.01	.10	.11	1.20	.86	9.30
24 hour day, 400 f.c. (1)	27	89.5	3.50	1.71	16.20	.08	.80	.17	1.60	1.96	18.50
24 hour day, 1200 f.c. (2)	22	74.4	2.10	11.87	46.30	.13	.51	.89	3.50	12.89	50.30
Same, except plants 10 days old (2)	46	75.2	1.50	11.37	45.90	.29	1.17	1.38	5.60	13.04	52.60
Greenhouse 2—2/7/1927											
12 hrs. sunlight + 12 hrs. artificial	18	75.5	2.0	13.38	54.60	.24	1.00	.35	1.40	14.00	57.00

## B. Stems and petioles only

Growth Conditions and Sampling Date of Control Plants	Wt. per Plant, Grams	% Moisture	Nitrogen % Dry Weight	Carbohydrates, % Wet and Dry Weights							
				Acid Hydrolyzable		Sucrose		Dextrose		Total Carbohydrate	
				Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Control 4/16/1928	23	91.4	—	1.19	13.85	.24	2.84	.54	6.24	1.97	22.93
24 hour day, 150 f.c.	37	92.7	1.34	0.79	10.78	.10	1.41	.31	4.24	1.20	16.43
24 hour day, 700 f.c.	34	91.7	0.73	0.92	11.14	.16	1.99	.71	8.64	1.79	21.77
24 hour day, 1300 f.c.	—	89.1	1.04	1.56	14.35	.20	1.83	1.06	9.76	2.82	25.94

(1). Exposed in the constant-light room at various distances from 2 1500-watt lamps without glass-water filter. Humidity 90 percent. Temperature 80° F.

(2). Grown in 1926 experiments in constant-light room with glass-water filter.

slightly lower level than in greenhouse plants, while at 700 foot candles (without a filter) the carbohydrates are at about the same level. The analysis of plants grown with daylight 12 hours supplemented by artificial light 12 hours from the gantry crane making a 24 hour day (Greenhouse 2, 1927) are included in this table. These plants showed an injury very similar to those growing under continuous artificial illumination in the constant-light room although not as severe. Tomato plants will not withstand a 24 hour day, 12 of which is sunlight. The rate of development of the injury, however, is decreased considerably by 12 hours of daylight in the combination. This is shown in text figure 11. The 24 hour all artificial light plant is more severely injured than the plant from Greenhouse 2. Both have been illuminated continuously since January 28, but the plant marked "House 2" received 12 hours of sunlight each day. Both plants have yellow leaves. The picture was taken on February 19, after the plants had been in the conditions 22 days. In table 10 it will be seen that total carbohydrates represent over 50 percent of the dry weight of leaves grown either on a combination of 12 hours daylight plus 12 hours artificial light, or on a 24 hour day of artificial light only at about 1300 foot candles. The total green weight of plant tissue at the end of seven days' exposure was as follows: Control greenhouse, 435 grams; 24 hour day, all artificial, 613 grams; 24 hour day, 12 of which was daylight, 497 grams. Twelve plants were used in each case. All plants grew approximately 4.5 inches in height during the experiment. It is evident, therefore, that the plants grow and increase in weight even in continuous illumination for short exposures. That they do not continue to do this is no doubt due to the breaking down of the mechanism of photosynthesis rather than to too great an accumulation of the products of this process.

To date no records have been found on the growth of tomato plants with continuous sunlight in the arctic regions. It would be interesting to know whether similar injuries develop in tomato plants grown under such natural conditions. The energy value in the constant-light room calculated at 0.3 gram calory per square centimeter per minute amounts to approximately 12,960 gram calories per month of 30 days. The total for the month of solar and sky radiation as published by the New York Observatory for June 1929 was approximately 11,903 gram calories. The two energy values are similar but as already pointed out the spectral distribution is in no way comparable. The glass-water filter in the constant-light room absorbs practically all radiation of wave length longer than 1400  $m\mu$  so that the total energy value of 12,960 gram calories includes only the visible region and the near infra-red of wave length shorter than 1400  $m\mu$ .

Work already mentioned (3) has shown that sunlight has a much higher percentage of the total energy value in the visible region than the tungsten filament lamp. Since the plant uses only the energy in the visible region



and near ultra-violet for photosynthesis it is probable that the total available energy in the constant-light room per month is less than June sunlight. There is a considerable difference in the constancy of sunlight as compared to artificial light. Sunlight varies widely from minute to minute whereas the main variation in the artificial light source is brought about by the slight voltage changes of the current supply causing only insignificant fluctuations in light intensity. These differences in the two light sources can not be considered as causal agents of the injury produced by continuous artificial illumination on plant tissue since it is not known whether continuous sunlight of similar intensity will produce a similar injury.

Guthrie (6) found that chlorophyll and carotinoids decreased in the leaves of tomato plants exposed to continuous illumination. The *a* to *b* chlorophyll ratios and the carotin to xanthophyll ratios were lowered. In the case of chlorophyll, *a* decreased faster than *b*. A brown pigment, associated with a state of disturbed metabolism within the plant, increased under these conditions. It is not known whether these facts are the cause of the breaking down of the plant or only associated with it. The fact that there is a shift in the *a* to *b* ratio is especially interesting since this is normally a constant under a great range of conditions.

It is interesting to note that 12 hours of daylight in the total 24 hour light exposure per day (Greenhouse 2) decreases the severity of the injury to tomato plants but does not entirely eliminate it. During the 1927 experiments an attempt was made in the constant-light room to produce an artificial light source comparable with sunlight in spectral distribution. Three 25-ampere white flame carbon arc lamps were used in one corner of the room with 22 1500-watt incandescent lamps uniformly distributed over the rest of the ceiling of the room. The glass-water filter was used to absorb the infra-red. Tomato plants were placed on the growing benches immediately under the arc lamps. The injury developed a little more slowly in this case but the final result was the same as had been found where all incandescent filament lamps had been used. The plants died in about four weeks. White flame arc lamps furnish a better light source for growing plants on account of the quality of radiation produced, but owing to the difficulty of maintaining these lamps they are at present impractical. When the arc is not protected against rapid oxidation by a glass globe the lamp must be trimmed frequently; in the present experiments at two hour intervals. When the glass globe is used to increase the life of the carbons cerium fluorid and other metallic salts from the cores of the carbon deposit on the inner wall of the globe and produce a rapid decrease in the light output. Four mercury vapor arcs in glass tubes were also used in this study along with 25 1500-watt filament lamps to increase the blue component in the light source. Probably on account of the low energy value of the output from the mercury lamps no visible benefit was observed either on tomato plants or other plants grown in the room.

It should be noted that on account of the extreme ultra-violet radiation produced by some arc lamps these lamps should not be used for growing plants without a glass filter, unless the possibility of injury has been carefully tested. The injury of the ultra-violet region beyond the limits of sunlight has been discussed in another paper (1). The intensity and wave length of the ultra-violet produced by open arc lamps depends upon the material mixed with the carbon or the material of the core in case of cored carbons, as well as upon amperage and other factors.

### **Chemical Composition of Various Species of Plants Grown Under Different Conditions**

#### *Chemical Composition of Cabbage*

Cabbage, variety Early Jersey Wakefield, was grown with various lengths of day in the 1924, 1925, and 1926 experiments. The chemical analysis of these plants is given in table 12. Where the plants were grown entirely with artificial light weight per plant increased with day length from five hours to 17 or 19 depending upon light intensity and temperature. Cabbage grew best at the higher temperature, 78° F., in 1925 on a medium light intensity, 970 foot candles. Plants growing on seven to 24 hour days produced heads. The best head was produced on a 17 hour day in the 1925 series. Five, seven, and 17 hour day cabbage grown with artificial light entirely are illustrated in text figure 12. All plants grown on day-lengths greater than seven hours of artificial light in this series produced warped and wrinkled leaves which later split in many places as they continued to grow. The seven hour day plant in its later stages of growth had some tendency to do this. The leaves of the five hour day plant remained perfectly smooth and flat during the experiment. As light intensity decreased the tendency to warp moved up into the longer day-length (19 and 24 hours). In the 1924 experiments plants grown at 300 foot candles without extra CO<sub>2</sub> produced smooth flat leaves on five, seven, 12, and 17 hour days. The mechanism of the splitting is apparently the unequal growth in different regions of the leaf lamina. It did not occur in any of the experiments where sunlight was used as part of the source of illumination. This may be due to the effect of a fixed source of light. Since cabbage leaves are almost perfectly rigid and are not able to orient themselves so as to vary the angle of incidence the rays from a fixed light source always strike parts of the leaf in the same place. This may result in a more rapid growth in certain spots of the leaf lamina, due to growth where food is most abundant. It appears also that the translocation of food in this species is mainly toward the midrib of the leaf and rarely from the center of the leaf toward the margin since otherwise the leaf margins would grow as rapidly as the rest of the leaf even if all photosynthesis took place in the center of the lamina. When illuminated with sunlight the angle of incidence is always shifting as the angle of the sun changes

TABLE 12. *Chemical Composition of Cabbage Plants*

Treatment	Wt. per Plant, Grams	Moisture %	Nitrogen-dry Weight Percentage		Carbohydrate-dry Weight Percentage				Carbohydrate-Nitrogen
			Soluble	Total	Acid Hydrolyzable	Sucrose	Dextrose	Total	
All artificial light, constant-light room. 1924. Sampled after 70 days. Temperature 78° F. Illumination 450 foot candles									
Control, greenhouse leaves only	781	91.4	—	2.64	12.68	1.85	13.95	28.48	10.8
5 hour day, leaves only	94	90.4	—	3.33	14.95	.53	5.36	20.84	6.3
7 hour day, " "	130	88.9	—	3.14	17.02	1.35	9.40	27.77	8.8
12 hour day, " "	472	91.4	—	2.71	18.75	1.31	10.92	30.98	11.3
17 hour day, " "	680	89.4	—	2.14	20.59	2.39	17.34	40.32	18.8
19 hour day, " "	715	85.6	—	1.22	41.75	1.28	8.65	42.0	17.2
24 hour day, " "	490	86.2	—	3.09	29.79	4.58	18.79	53.16	17.2
All artificial light, except G.H. 1 and G.H. 2. 1925. Sampled after 57 days. Temperature 78° F. Illumination 900 foot candles (1)									
Control, greenhouse	589	90.1	.72	2.35	16.22	3.53	17.20	36.95	15.6
5 hour day	362	92.5	1.94	3.36	12.65	1.42	11.46	25.53	7.5
7 hour day	379	92.8	1.71	2.57	11.73	1.28	16.48	29.49	10.4
12 hour day	476	92.8	1.26	2.52	10.24	1.58	13.09	24.91	9.9
17 hour day	907	93.0	1.24	2.47	12.22	3.07	21.57	36.96	15.0
19 hour day	1032	92.0	1.13	2.44	9.30	2.50	25.09	36.89	15.0
Greenhouse 1	768	86.9	.33	1.25	27.47	2.25	12.80	42.52	34.0
Greenhouse 2	893	85.1	.41	1.17	34.96	3.40	13.41	51.77	44.0
All artificial light except G.H. 1 and G.H. 2. 1926. Sampled after 65 days. Temperature 68° F. Illumination 1200 foot candles (1)									
Control, greenhouse	710	90.2	.70	2.03	15.36	2.53	21.21	39.10	19.4
5 hour day	248	91.7	1.56	3.53	10.41	—	11.23	21.64	6.1
7 hour day	485	91.2	1.25	3.01	12.86	—	19.09	31.95	10.5
12 hour day	414	90.2	1.74	3.30	8.98	—	16.97	25.95	7.9
17 hour day	722	90.7	1.04	2.12	14.10	2.97	27.06	44.13	20.8
19 hour day	633	86.1	.63	1.43	26.18	5.22	23.29	54.69	38.0
24 hour day	654	89.7	.99	2.15	20.61	3.43	22.42	46.46	21.5
Greenhouse 1	695	87.5	.71	1.92	15.06	3.56	20.80	39.42	20.6
Greenhouse 2	772	87.6	.84	1.68	29.96	4.11	21.05	55.12	33.0

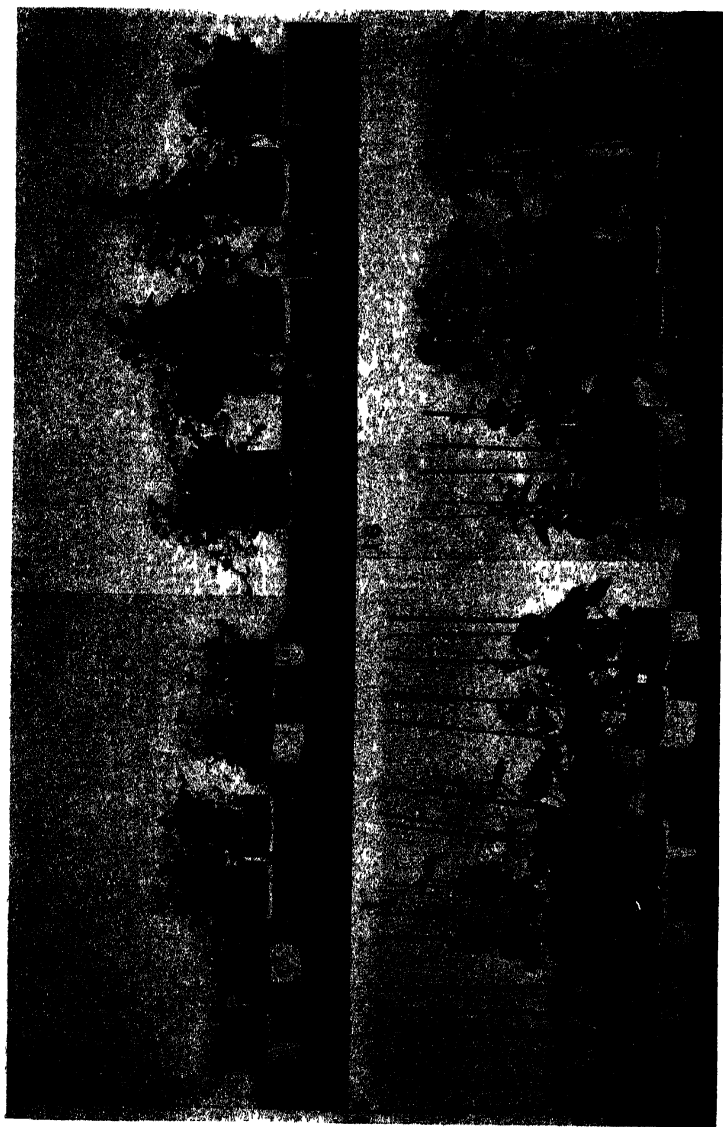
(1). Whole aerial portion analyzed.

resulting in different regions of the leaf lamina being illuminated more strongly at different times:

The percentage total carbohydrate in cabbage plants increases, and percentage total nitrogen decreases with day length from a five to a 19 hour day as shown in table 12. Percentage carbohydrates usually decreases on continuous illumination. The plant grows well on continuous illumination but reaches maximum tissue production on a day length of about 19 hours. As compared with control plants growing in the greenhouse with normal daylight during the period of the experiments both the carbohydrates and weight per plant are generally higher when normal light is supplemented by six hours of artificial light at night, making about an 18 hour day. There is a further increase in these two values in Greenhouse 2 which was given both additional light and carbon dioxide. The percentage composition of simple carbohydrates in cabbage was more than doubled on a 19 hour day as compared to a five hour day. The caloric value of such a food is greatly increased by growing it on a long day. This plant, normally considered as only a filler in an animal diet or at best a source of mineral salts and vitamins can be grown so that it has considerable fuel value as well.

#### *Red Clover*

Red clover grows exceptionally well on an 18 hour day in the gantry crane greenhouse, especially when the  $\text{CO}_2$  concentration is increased. It was grown from seed to flower in the brief period of 38 days, in both the 1925 and 1926 series. Text figure 13 shows red clover plants flowering on April 9 in both Greenhouse 1 and 2 from seed planted February 28, 1925. Text figure 14 shows the same series on May 8 when the plants were 69 days old. A remarkably good crop of clover hay was produced in Greenhouse 2 in this brief space of time. It would take two seasons' growth to produce this in ordinary agricultural practice. The control plants did not flower during the experiment. The plants grew much better in the 1925 series at  $78^\circ$ . The 1926 series with a lower temperature,  $68^\circ$ , and a higher light intensity on the 24 hour day gave a much poorer growth. The control and Greenhouse 2 plants grown in 1927 were started a month earlier (January 28) and on account of the lower solar intensity in February did not grow as rapidly. While clover grows well even with continuous illumination the 24 hour day plant is in general no better than an 18 hour day plant. Carbohydrates and weight per plant at favorable temperatures are both increased by supplementing daylight with six hours of artificial light from the gantry crane. The chemical analyses of plants grown in 1925, 1926, and 1927 are given in table 13. Additional carbon dioxide (Greenhouse 2) produces a further increase in both weight and total carbohydrates.



TEXT FIG. 13. Red clover. The two pots of plants at the left were grown in the control greenhouse, the two center with 6 hours of artificial light supplementing daylight and with about ten times the normal concentration of carbon dioxide (gantry crane greenhouse). The two at right received the same illumination but no gas. Age from seed, 40 days. TEXT FIG. 14. Three of the same pots of clover shown in FIG. 13 with the 24 hour day plant added. The latter was grown in the constant-light room with artificial light only. Age from seed, 69 days. TEXT FIG. 15. Cucumbers. Two pots at left, grown in control greenhouse. Two at center in gantry crane house with daylight plus 6 hours of artificial light each night plus ten times the normal carbon dioxide. Two at right same as center except no extra gas.

TABLE 13. *Chemical Composition of Red Clover. Whole Aerial Portion\**

Treatment	Wt. per Plant, Grams	Moisture %	Nitrogen-dry Weight Percentage		Carbohydrates-dry Weight Percentage				Carbohydrate Nitrogen
			Soluble	Total	Acid Hydrolyzable	Sucrose	Dextrose	Total	
Red clover, 1925. Age 76 days									
Control.....	1.1	76.6	.87	3.44	9.61	2.28	1.86	13.75	4.00
Greenhouse 1†.....	6.0	78.2	.77	2.73	11.80	2.50	2.66	16.96	6.4
Greenhouse 2†.....	9.2	70.6	.38	1.96	16.36	2.99	2.70	22.05	11.2
24 hour day†.....	10.6	75.8	.56	2.44	12.90	2.01	2.61	17.52	7.2
Red clover, 1926. Age 71 days									
Control.....	2.1	85.3	.46	3.18	12.25	2.04	3.52	17.81	5.6
Greenhouse 1†.....	2.2	84.4	.47	2.88	11.49	1.93	4.12	17.54	6.0
Greenhouse 2†.....	3.9	81.2	.27	1.76	19.14	2.30	5.84	27.28	15.6
24 hour day†.....	2.2	77.1	.28	1.08	9.28	.84	2.37	12.49	11.4
Red clover, 1927. Age 66 days									
Control.....	0.6	81.5	.62	2.90	4.92	2.30	1.44	8.66	3.0
Greenhouse 2 1927† (†).....	5.9	80.0	.39	2.13	15.53	4.86	4.58	24.97	11.6
24 hour day†.....	5.6	76.0	.66	2.12	14.34	3.90	3.84	22.08	10.4
24 hour day—24-hour night†.....	5.7	83.0	.42	2.48	13.15	4.14	2.90	20.19	8.1

\* Control plants grown in ordinary greenhouse.

Greenhouse 1, grown with daylight supplemented by 6 hours artificial light from crane.

Greenhouse 2. Same as above except extra CO<sub>2</sub> about .3 percent.24 hour day, grown with artificial light only in light room with CO<sub>2</sub> at about .3 percent.

† Flowering.

‡ Grown with 12 hours daylight plus 12 hours of artificial light and with extra CO<sub>2</sub>.

TABLE 14. *Chemical Composition of Soy Beans, 1925. Age 40 Days. Whole Aerial Portion\**

Treatment	Wt. per Plant, Grams	Moisture %	Nitrogen % Dry Weight		Carbohydrate % Dry Weight				Carbohydrate Nitrogen
			Soluble	Total	Acid Hydrolyzable	Sucrose	Dextrose	Total	
Mandarin, control†	7.8	83.0	1.07	3.71	19.22	2.06	1.38	22.7	6.1
Mandarin, G.H. 1†	35.8	80.7	.43	2.97	19.33	2.00	2.69	24.0	8.1
Mandarin, G.H. 2†	32.1	77.9	.23	1.81	26.12	1.81	3.36	31.3	17.2
Peking, control†	3.8	83.9	1.30	4.12	15.03	2.06	.76	17.9	4.4
Peking, G.H. 1	19.5	82.5	.75	3.32	15.97	2.10	1.38	19.5	5.9
Peking, G.H. 2	38.1	79.3	.35	2.19	19.54	2.35	3.59	25.4	11.6
Tokio, control†	11.6	84.3	1.08	3.73	13.63	1.66	.81	17.1	4.6
Tokio, G.H. 1	30.8	82.5	.70	3.79	15.22	2.37	.68	18.3	4.9
Tokio, G.H. 2	52.1	81.1	.33	2.57	16.75	2.57	3.92	23.2	9.1
Biloxi, control†	14.2	82.4	.93	3.81	16.40	1.72	.91	19.0	5.0
Biloxi, G.H. 1	52.8	80.5	.70	3.19	14.86	2.54	1.63	19.0	6.0
Biloxi, G.H. 2†	50.8	75.0	.22	1.50	30.84	1.82	2.28	34.9	23.0

\* Grown in control or ordinary greenhouse at 78° C. Sampled 4/8/25.  
 Greenhouse 1, greenhouse plus 6 hours artificial light from gantry crane.  
 Greenhouse 2, same as above except extra CO<sub>2</sub>.  
 † Fruiting or flowering.

*Soy Bean*

Four different varieties of soy beans were grown in the control and gantry crane greenhouses in the 1925 series, Mandarin, Peking, Tokio and Biloxi. Garner and Allard (4) observed that these varieties flowered on the following dates when grown outdoors in Washington, D. C.: Mandarin on June 15; Peking on July 10; Tokio, August 1; and Biloxi on September 1. In the present experiments Mandarin flowered and set fruit both in the control and in the greenhouses with supplementary light. Peking flowered and set fruit only in the control while Tokio did not set fruit under any of the conditions but flowered in the control greenhouse. Biloxi flowered and set a few fruit in the control and also in Greenhouse 2 with both extra light and carbon dioxid during the last few weeks of the experiment. The last observations were made on May 29, 1925, when the plants were 91 days old. It is not known whether the other varieties would have flowered in other conditions if the experiment had been continued. Since only a few plants were grown and fruiting does not occur in all individuals, the data on day length at which these varieties will flower are not conclusive. Biloxi, Mandarin and Tokio varieties grew to a height of over 40 inches during this time. The data from the chemical analysis of the four varieties are presented in table 14. It will be observed that weight per plant and total carbohydrates in general increase on the longer day in Greenhouse 1 and 2, and again in House 2 as compared with House 1. Total and soluble nitrogen and moisture decrease in the same direction.

*Cucumber*

Cucumber plants were grown with additional light and carbon dioxid in the 1925, 1926, and 1927 experiments. This plant was greatly favored by the higher temperatures in the 1925 and 1927 experiments. As compared to the control plants it produced more than twice the amount of tissue with additional light and gas. The increased rate of growth with additional light and gas is shown in text figures 15 and 16. The first picture was taken when the plants were one month old and the second, nine days later. The plants growing in Greenhouse 2 in 1925 attained a height of 36 inches in 30 days from the time the seed was planted. Fruits were setting in 35 days. In the last few weeks of the experiment the leaves yellowed considerably in Greenhouse 2. It was thought that this might be due to a shortage of nitrate nitrogen were carbohydrates were being built up too rapidly since leaves of the control plants remained dark green in color. In the 1926 and 1927 experiments some of the plants were given 2.5 to five grams of sodium nitrate each week for four weeks. This was effective in maintaining a dark green leaf color in all of the plants growing in the different conditions. Such high concentrations of nitrate stunted the growth of plants in the control as compared with Greenhouse 2 in which the plants were grown with extra light and carbon dioxid. The weight



TABLE 15. *Chemical Composition of Cucumber Plants. Whole Aerial Portion*

Treatment and Sampling Date	Wt. per Plant, Grams	Moisture %	Nitrogen—Dry Weight %		Carbohydrate—Dry Weight %				Carbohydrate Nitrogen
			Soluble	Total	Acid Hydro-lyzable	Sucrose	Dextrose	Total	
Greenhouse control*—4/14/1925.....	134	90.4	1.11	3.95	15.5	2.45	.99	18.94	4.8
Greenhouse 1*—4/14/1925 (3).....	244	91.1	.50	2.64	15.1	1.94	2.54	19.56	7.5
Greenhouse 2*—4/14/1925 (4).....	315	89.4	.25	2.01	26.3	1.66	3.94	31.88	15.8
Greenhouse control†—4/21/1926.....	62	91.4	1.00	4.38	15.1	2.04	1.59	18.74	4.3
Greenhouse 1*—4/21/1926 (3).....	93	92.4	1.24	4.71	14.2	1.30	1.62	17.10	3.7
Greenhouse 2*—4/21/1926 (4).....	210	88.5	.27	1.82	25.5	4.33	4.89	34.74	19.0
Greenhouse control* with NaNO <sub>3</sub> —4/21/26 (1).....	60	87.8	1.35	3.93	13.8	2.08	1.94	17.80	4.6
Greenhouse 2 with NaNO <sub>3</sub> —4/21/1926 (1) (3).....	42	90.7	1.88	5.52	11.1	1.85	1.46	14.41	2.6
Greenhouse control—3/31/1927.....	50	90.7	1.50	5.35	13.4	2.57	1.50	17.45	3.3
Greenhouse control with NaNO <sub>3</sub> —3/31/27 (2).....	16	90.1	2.12	4.94	9.1	2.82	.81	12.70	2.6
Greenhouse 2—3/31/27—24-hour day (5).....	361	89.7	.48	2.32	27.7	1.65	4.65	29.02	12.6
Same, with NaNO <sub>3</sub> (5) (2).....	213	90.7	1.50	4.40	14.2	2.68	2.36	20.20	4.6

† Flowers only.

\* Flowers and fruit.

(1). Received extra nitrate at rate of 5 grams  $\text{NaNO}_3$  per week for 4 weeks.(2). Received extra nitrate at rate of 2.5 grams  $\text{NaNO}_3$  per week for 4 weeks.

(3). Grown with 12 hours sunlight plus 6 hours of artificial light from crane making an 18 hour day. Temperature 78° F. in 1925 and 68° F. in 1926.

(4). Same as (3) except with higher concentration of  $\text{CO}_2$  about .3 percent.

(5). Grown with 12 hours sunlight plus 12 hours of artificial light from crane making a 24 hour day. Temperature 78° F.

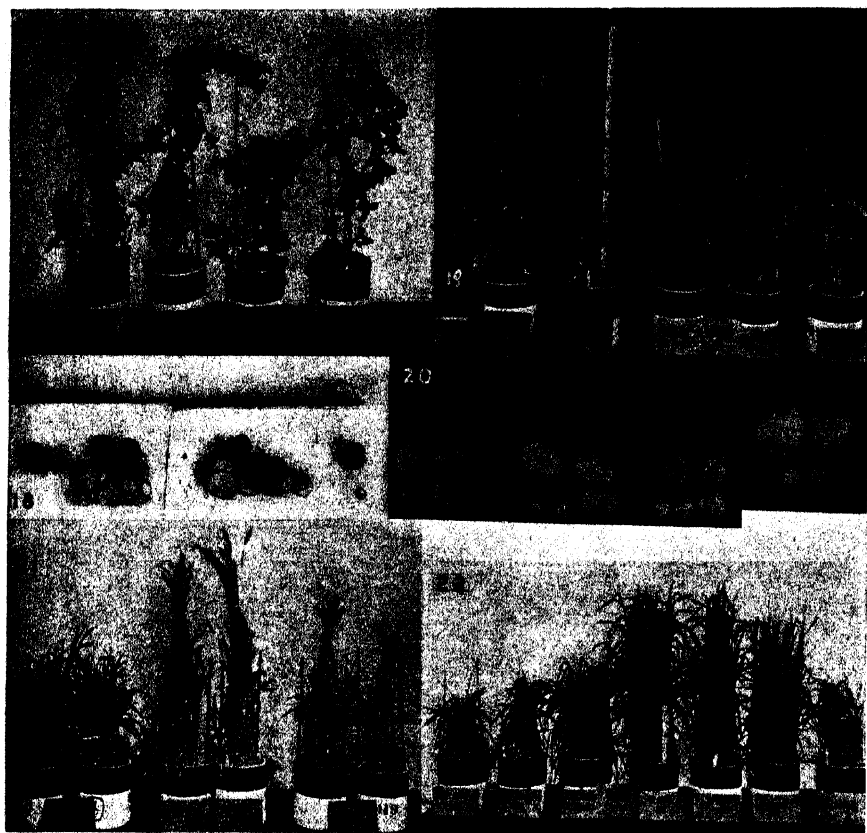
per plant as well as the chemical analysis of these plants is given in table 15. It will be observed that nitrate greatly reduced both the weight per plant and the total carbohydrate produced in most cases.

In contrast to the results with salvia, cucumber was found to absorb nitrates rapidly enough to increase the total nitrogen content considerably. This was especially true in case of the plants growing with extra light and carbon dioxid where total nitrogen was low and carbohydrate high. The controls did not absorb nitrate appreciably, but they were already high in this fraction. The application of nitrate to the soil in the case of cucumber results in a definite increase in total nitrogen where the nitrogen reserve has been depleted due to accumulation of carbohydrate material. There was no outstanding effect on flowering and fruiting where plants were fed additional nitrate except where leaf injury developed due to the toxicity of high concentration of this salt. Hand pollination was used to induce setting of fruit. Too few plants were grown in these experiments to judge relative yields accurately but plants grown with additional light and CO<sub>2</sub> produced larger fruit. Total carbohydrates and weight per plant increased with both additional light and with higher concentrations of carbon dioxid.

#### *Potato*

Potatoes of the Irish Cobbler variety were grown both in the gantry crane greenhouse and in the constant-light room in 1925, 1926, and 1927. The results of these experiments are of especial interest since the potato is known to be a low temperature plant. The grains, barley and spring wheat, are also commonly considered to be low temperature plants. Grains in general were found to grow well and yield well in these experiments, even at a comparatively high temperature (78° F.) if additional light and carbon dioxid is supplied. In general, many species of plants will give higher yields at a higher temperature when additional light and carbon dioxid are supplied. Tuber production in the potato in contrast with this seems limited to a low temperature. High temperature produced weak-stemmed bushy plants and little or no tuberization, although weight produced of the aerial portion at high temperature was usually greater than at low temperature. Text figures 17 and 18 show potato plants grown with a high temperature of 78° F. in 1925 together with the respective yields. Tubers marked + in text figure 18 are mother-tubers. Figures 19 and 20 show potato plants grown at a low temperature of 68° F. in 1926 and the respective yields.

Various opinions have been recorded on the effect of both day length and temperature upon tuberization of the potato. Garner and Allard (4) have given a considerable discussion of the literature on the subject and have added their own observations. They found that McCormick potatoes growing in a greenhouse during the summer did not tuberize on a long day. The temperature was comparatively very high in these experiments.



TEXT FIG. 17. Irish Cobbler potato plants. Left to right, grown with daylight plus 6 hours of artificial light each night from the gantry crane, the same except extra carbon dioxide, control on normal greenhouse conditions, and 24 hour day or continuous artificial illumination in the constant-light room. Temperature 78° F. TEXT FIG. 18. Shows the yield of tubers from the plants in Fig. 17. The tubers marked + are all mother tubers. TEXT FIG. 19. Same series as FIG. 17 except grown at a lower temperature, 68° F. Arranged in different order from FIG. 17. Left to right, control, gantry crane + extra carbon dioxide (House 2), the same except no extra gas (House 1), 24 hour day all artificial light and greenhouse plus scrubbed flue gas as a source for carbon dioxide. The plants with long days and extra gas flowered especially well. TEXT FIG. 20. Shows the yield of tubers from plants grown at low temperature, FIG. 19. Long days, including continuous illumination greatly favors high yield of tubers at low temperature. TEXT FIG. 21. Barley grown at a high temperature, 78° F. The two pots of plants at left were grown in the control greenhouse, two at center in the gantry crane greenhouse with daylight plus 6 hours supplementary lighting plus a higher concentration of carbon dioxide, the two right same as center except no extra gas. TEXT FIG. 22. Barley grown at a low temperature, 68° F., in the constant-light room on 5, 7, 12, 17, 19, and 24 hour days. The plants marked control at right were grown under ordinary greenhouse conditions at the same temperature.

When grown outdoors and exposed to different day lengths, tuberization of this variety increased with day length up to a 13 hour day but fell away slightly on full day length. They conclude from these experiments that a very long day tends to direct the activities of the plant toward vegetative development. With a somewhat shorter day the tendency is toward sexual reproduction and moderate tuber formation and with further shortening of the day seed development fails and there is a tendency toward tuber formation. McClelland (15) found that weight of tops increased with day length in three varieties of potatoes. Tuberization varied with variety. Irish Cobbler produced a greater weight of tubers on a short day of ten hours as compared to a 15 hour day. Maximov (14) concluded, from his experiments, that all varieties of Russian potatoes increased tuberization with shortening of the days. Bushnell (2) found, along with many previous workers, that size of leaf and amount of tuberization decreased with increasing temperature. This, he concluded, was due to the high respiration rate at higher temperatures which used up carbohydrates too rapidly to admit of any storage in tubers.

The results of a chemical analysis of the whole aerial portions of potato plants grown in the 1926 and 1927 experiments are given in table 16. Photographs of both plants and tubers produced in the 1925 and 1926 series are shown in figures 17, 18, 19, and 20. The weight per plant of the aerial portion was greater in the high temperature series grown in 1927 (78° F.). Plants grown in the gantry crane Greenhouse 2 produced the greatest weight of top in 1927. This was an 18 hour day with additional carbon dioxid. Tuberization was very poor. The second highest in weight of tops was the 24 hour day grown in the constant-light room in 1927 at 78° F. This plant produced only one tuber about one-half inch in diameter. The greatest yields of tubers were produced by the long day plants of the cool temperature series, Greenhouse 2 and 24 hour day plants. These plants produced medium to low weight of tops. Total nitrogen was low in the 1926 low temperature series as compared with the high temperature series while carbohydrates were slightly higher. The only conclusion which can be made from these data is that this variety of potato utilizes much of the carbohydrate produced at low temperature for tuber production and very little for growth of the aerial portion. With increasing day length and high light intensity more carbohydrates are formed and consequently more and larger tubers are built up. At higher temperatures much of the carbohydrate produced is diverted toward producing growth of the aerial portion and little is available for tuber building. Increasing light intensity and day length at high temperatures results only in producing more top. Ecologically this should mean that the largest yields of potato tubers are produced in northern latitudes where air temperature is cool, day length long, and light intensity high, assuming that soil and other factors remain the same. This is generally known to be the case, high

TABLE 16. *Chemical Composition of Potato Plants. Irish Cobbler Variety*

Treatment and Date Sampled	Wt. of Tops per Plant, Grams	Moisture %	Nitrogen, % Dry Weight		Carbohydrate, % Dry Weight				Tuber Yield
			Soluble	Total	Acid Hydro- lyzable	Sucrose	Dextrose	Total	
Control greenhouse—4/22/1926—68° F.	271	89.2	.68	3.29	15.8	2.82	5.00	23.6	Fair
Greenhouse 1—4/22/1926—68° F.*	495	89.7	.44	2.76	16.1	3.13	5.84	25.1	Fair
Greenhouse 2—4/22/1926—68° F.*	323	89.5	.27	1.80	14.2	1.50	5.69	21.4	Very good
24 hour day—4/22/1926—68° F.*	197	82.8	.17	.89	9.0	—	6.90	—	Very good
Control greenhouse—4/1/1927—78° F.	246	92.0	2.13	5.38	8.3	2.00	1.50	11.8	Fair
Greenhouse 2—4/1/1927—78° F.*	862	89.7	.88	3.41	13.1	3.31	2.92	19.3	Poor
Greenhouse 1—4/1/1927—68° F.	307	90.8	1.75	5.24	9.6	3.38	1.31	14.1	Fair
24 hour day—4/1/1927—78° F.*	597	89.1	1.37	3.75	12.1	2.74	2.19	17.0	Poor

\* Flowering.

Greenhouses 1 and 2 in 1926 received 6 hours artificial light from crane.

Greenhouses 1 and 2 in 1927 received 12 hours artificial light from crane.

Greenhouse 2 received increased concentration of CO<sub>2</sub> (about .3 percent) in 1926 and 1927.24 hour day plant grown with artificial light entirely with increased CO<sub>2</sub>.

latitudes producing much greater average yields of potatoes than equatorial, and higher altitudes greater yields than low altitudes.

### *Small Grains*

Barley, wheat, and oats were grown in the control and gantry crane houses during most of the experiments. Barley was also grown on various day lengths in the constant-light room in the 1926 series. These grains were found to grow well even at a high temperature (78° F.) when both additional light and carbon dioxid were given. The grain yields in grams per pot of oats, barley and spring wheat (blue stem variety), were as follows:

	Oats	Barley	Wheat
Control greenhouse.....	3.46	4.00	3.78
Greenhouse 1.....	1.00	3.16	4.42
Greenhouse 2.....	2.30	20.50	9.53

A photograph of barley grown at a high temperature in the 1925 gantry crane series is reproduced in text figure 21. Plants grown at a low temperature in the constant-light room and control greenhouse in 1926 are shown in figure 22.

The work of Walster (20) has already been mentioned. In brief he found that barley grown at a high temperature with high nitrate supply produced weak stems and a prostrate type of growth. The prostrate habit of barley grown at 20° C. in Walster's work was no doubt produced by the low light intensity and short day conditions under which he worked. In the present experiments barley grew well and produced sturdy stems with comparatively high yields when grown continuously at 25° C. on day lengths of 17 to 19 hours (1925 series, fig. 21). When grown in the constant-light room on short days of five to 12 hours at 20° C. with an abundant supply of nitrogen a prostrate growth was produced. This is shown in text figure 22, a photograph of the 1926 series.

The results obtained from the chemical analysis of barley is tabulated in table 17 while similar data for oats and wheat are given in table 18. The weight per plant of barley increases with day length up to a 19 hour day. Total carbohydrates increase in the same way up to a 24 hour day, while total nitrogen decreases with day length. Addition of nitrate to the soil makes little or no difference in the total percentage of nitrogen in the barley plant. When total nitrogen percentage in the plant is brought to a low value by an increase in carbohydrates, due to long days and extra CO<sub>2</sub> concentration, the nitrogen fraction is changed very little, if any, by the addition of sodium nitrate to the soil of barley plants. This plant is apparently able to limit nitrogen intake independent of the concentration of nitrate in the soil.

In table 18 the analytical data for oats and spring wheat grown with

TABLE 17. *Chemical Composition of Barley. 30 Days Old, from Seed. 1926. Aërial Portion*

Treatment	Wt. per Plant, Grams	Moisture %	Nitrogen, % Dry Weight		Carbohydrate, % Dry Weight				Carbohydrate Nitrogen
			Soluble	Total	Acid Hydrolyzable	Sucrose	Dextrose	Total	
Control greenhouse	1.2	89.1	1.62	5.05	10.52	3.94	3.06	17.52	3.4
5 hour day (1)	1.2	91.0	1.89	5.42	—	—	—	—	—
7 hour day (1)	1.9	90.7	1.84	5.10	8.06	3.57	3.13	14.76	2.9
12 hour day (1)	2.8	90.9	1.87	4.89	10.60	3.23	2.66	16.49	3.4
17 hour day (1)	4.5	86.7	.81	2.93	16.57	5.58	7.40	29.55	10.1
19 hour day (1)	4.7	82.9	.44	1.64	16.22	8.80	9.49	34.51	21.0
24 hour day (1)	2.9	84.2	.77	2.70	17.71	8.55	10.32	36.58	13.6
Greenhouse 1 (2)	1.5	84.6	1.24	3.85	16.41	7.17	6.61	30.19	7.8
Greenhouse 2 (3)	4.3	83.5	.69	2.69	15.95	4.87	8.50	29.32	10.9
Barley with NaNO <sub>3</sub> added, * 5 grams per pot per week for 2 weeks. 30 days old, from seed. 1926.									
Control greenhouse	1.7	88.6	1.86	2.95	9.98	4.29	2.89	17.16	
Greenhouse 1 (2)	1.4	84.0	1.52	4.14	14.96	6.19	5.07	26.22	
Greenhouse 2 (3)	3.4	81.8	.93	3.21	18.47	10.54	5.35	34.36	
Barley with and without extra nitrate. * Forty days old, from seed. 1927									
Control greenhouse	1.2	83.2	1.25	3.57	13.67	6.00	4.75	24.42	6.8
Same—extra nitrate	1.2	82.2	1.35	3.71	13.52	6.17	4.43	24.12	6.5
Greenhouse 2 (3)	3.5	79.3	.92	2.51	19.80	5.55	1.88	27.23	10.8
Same—extra nitrate	4.0	78.2	.78	2.20	18.14	8.08	2.16	28.38	12.8

\* Received solution at the rate of five grams per pot of NaNO<sub>3</sub> each week for three weeks starting when plants were two weeks old in 1926 series. In 1927 series each pot received 2.5 grams of NaNO<sub>3</sub> each week for three weeks starting when the plants were three weeks old.

(1). Grown in constant-light room with artificial light only plus extra carbon dioxide.

(2). Grown in greenhouse with six hours supplementary light each night from crane.

(3). Same as (2) except with extra carbon dioxide in 1926 experiments. In 1927 experiments greenhouse 2 received 12 hours supplementary light each night from crane.

TABLE 18. *Chemical Composition of Oats and Wheat. Aerial Portion*

Treatment	Wt. per Plant, Grams	Moisture %	Nitrogen, % Dry Weight		Carbohydrate, % Dry Weight			
			Soluble	Total	Acid hydro-lyzable	Sucrose	Dextrose	Total
Oats, 1926, 51 days old								
Control greenhouse.....	3.5	84.4	.39	1.80	16.13	6.86	3.53	26.52
Greenhouse 1.....	1.1	77.7	.59	1.96	13.00	8.74	2.45	24.19
Greenhouse 2.....	9.0	74.6	.12	.64	13.24	26.27	4.00	43.51
Wheat, variety blue stem, 1926, 48 days old								
Control greenhouse. No heads produced.....	3.4	86.4	.93	3.42	13.56	2.73	2.87	19.16
Greenhouse 1. Straw only.....	3.3	76.4	.47	2.15	15.46	8.07	3.26	26.79
Greenhouse 2. Straw only.....	6.3	68.2	.25	1.16	15.50	20.29	1.88	37.17
24 hour day. Straw only.....	1.8	64.6	.29	1.15	18.11	14.13	2.93	35.17
Greenhouse 1. Heads only.....	.6	71.3	.60	2.18	25.43	28.07	6.79	60.29
Greenhouse 2. Heads only.....	2.1	64.7	.51	2.11	40.53	10.83	3.12	54.48
24 hour day. Heads only.....	.5	61.5	.26	1.26	30.01	14.14	3.80	47.95

Greenhouse 1 received six hours supplementary light each night from crane.

Greenhouse 2 received same illumination and additional carbon dioxide.

24 hour day plants grown in constant-light room.



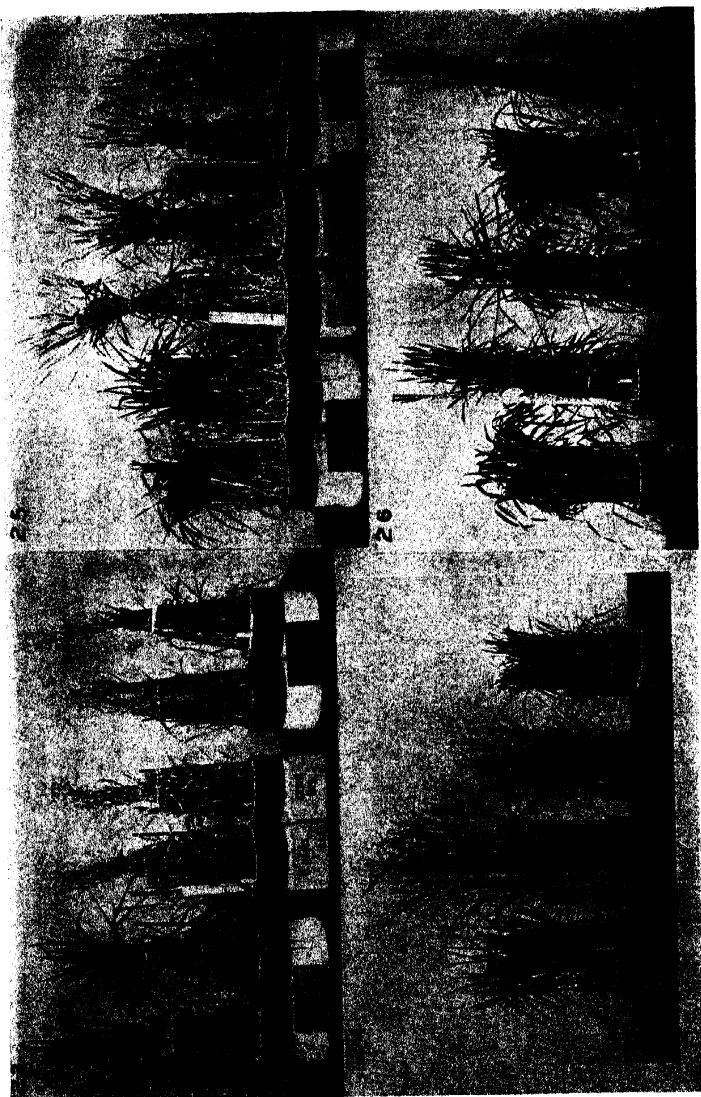
TABLE 19. *Chemical Composition of Tobacco and Ragweed, 1925. Aerial Portion*

Treatment	Wt. per Plant, Grams	Moisture %	Nitrogen, % Dry Weight		Carbohydrate, % Dry Weight			
			Soluble	Total	Acid Hydro-lyzable	Sucrose	Dextrose	Total
Tobacco, under conditions 38 days								
Control greenhouse.....	866	88.3	.20	1.12	21.8	2.85	7.14	31.8
Greenhouse 1*.....	1230	86.6	.22	1.14	24.8	3.65	7.67	36.2
Greenhouse 2*.....	1295	84.2	.15	.51	33.6	3.92	8.45	46.0
Constant-light room*.....	817	85.7	.21	1.28	29.0	3.62	6.47	39.1
Ragweed, under conditions 60 days								
Control greenhouse*.....	260	81.1	.73	3.27	13.9	2.14	1.07	17.13
Greenhouse 1.....	333	80.2	.48	2.54	15.1	3.24	1.73	20.03
Greenhouse 2.....	598	72.5	.11	.88	17.9	12.98	1.99	32.89

\* Flowering and fruiting.

Greenhouse 1 received daylight plus six hours additional light from gantry crane each night.

Greenhouse 2 received same illumination as 1 but had about 10 times the normal CO<sub>2</sub> concentration.Constant-light room received continuous artificial illumination plus additional CO<sub>2</sub> as in 2.



TEXT FIG. 23. Clydesdale oats grown at 78° F. Two at left grown in control greenhouse, two at center from gantry crane house with higher concentration of carbon dioxide, two at right same as center except normal carbon dioxide concentration. All plants 45 days old from seed. TEXT FIG. 24. A series of oats similar to FIG. 23 except grown at low temperature, 68° F. The plants at right marked "flue gas" were grown under normal greenhouse conditions except with carbon dioxide concentration about ten times normal. The gas was produced by scrubbing flue gases. All plants 34 days old from seed. TEXT FIG. 25. Blue stem, spring wheat. Two pots at left grown in control greenhouse, two at center with 6 hours supplementary light plus higher carbon dioxide concentration, two at left same as center except normal carbon dioxide concentration. Plants 47 days old from seed. Temperature 78° F. TEXT FIG. 26. Blue stem wheat similar to FIG. 25 except grown at 68° F. Plants 48 days old from seed. Left to right control, House 2 received 6 hours supplementary lighting plus higher concentration of carbon dioxide from steel cylinder.

and without additional light and carbon dioxid is listed. Those plants receiving both additional light and carbon dioxid weigh much more and have greater amounts of carbohydrates as compared to the control plants growing in the greenhouse. The oat plants in the control house were just coming into head when sampled while those in Greenhouse 1 and 2 had been in head several days. The heads were discarded before sampling for analysis. The control wheat plants showed no sign of heading when sampled while those in Greenhouse 1 and 2 had been in head for some time.

Text figure 23 illustrates oat plants (Clydesdale variety) grown at a high temperature in 1925 (78° F.). Figure 24 is a similar series grown at a low temperature in 1926 (68° F.). Those grown at the low temperature have stiff straw and small leaves while those grown at the high temperature have larger and more succulent leaves with a more flexible straw. Those grown with extra light only (gantry crane house 2) in 1926 were shorter and weaker than those grown in the same house in 1925.

Two varieties of winter wheat were grown, Turkey Red and Hybrid 128, also spring wheat of the Blue Stem variety. Wanser (21) reported that winter wheat required a critical photo-period for jointing and a different photo-period for heading. Schafer, Gaines and Barbee (18) state that Hybrid 128 does not head when planted later than March 11, while Turkey Red planted in April will joint in October while Hybrid 128 will not.

In these experiments Hybrid 128 always produced a few heads on an 18 hour day in the gantry crane house and on a 24 hour day with continuous artificial light. Turkey Red did not head under these conditions but formed dense mats of vegetative growth. Spring wheat was always favored by long days and was grown from seed to head in 31 days in both the gantry crane house 2 and constant-light room in the 1925 experiments. Photographs of these plants are shown in text figures 25, 26, 27, and 28.

#### *Other Plants Grown*

Several other species of plants were grown in the gantry crane houses with supplemented light. Of these only tobacco and ragweed (*Ambrosia artemisiifolia*) were analyzed. This data is presented in table 19, while photographs of these plants are reproduced in text figures 29 and 30. The ragweed is a short day plant. It flowered on April 21 after 54 days in the control greenhouse at a height of about 19 inches while it remained vegetative and did not flower in the gantry crane houses on an 18 hour day reaching a height of 40 inches and a weight more than twice as great as the control plants. The weight per plant and total carbohydrates increased with additional light and again with additional gas both with tobacco and ragweed plants. Total nitrogen decreased. This has been found to be true in general with most plants grown in these experiments.

Several ornamental plants such as roses, sweet peas, snapdragon, petunia and nasturtium grew and flowered remarkably well with additional light



TEXT FIG. 27. Winter wheat, Hybrid 128, showing the tendency to head when grown with daylight plus 6 hours of artificial light plus additional carbon dioxide (House 2) or in the constant-light room under continuous illumination. This variety did not head in the control greenhouse or in House 1 with 6 hours of artificial light and the normal carbon dioxide concentration. Plants 66 days old from seed. TEXT FIG. 28. Winter wheat, Turkey Red variety, did not head under any of the conditions. TEXT FIG. 29. Ragweed (*Ambrosia artemisiifolia*) showing the flowering on the short days in the control greenhouse and tendency to remain vegetative in Houses 1 and 2 where the plants received 6 hours additional illumination each night. Plants in House 2 receiving higher concentration of carbon dioxide grew more rapidly than those in

and carbon dioxide. Carnations did not respond to additional light and gas in the 1927 experiments. This may have been due to the comparatively high temperature (78° F.) since carnations are known to grow best at a low temperature. Hoosier Beauty, Premier, and roses of the rambler type all flowered profusely with additional light and carbon dioxide. Hoosier Beauty and Premier sent up new canes from the root-stock which produced clusters of two or three flowers in less time than was required for flowers to develop from existing canes in the control plants at this season of the year. One of the common effects of these conditions was the production of two or three large blooms on a single cane at one time. This effect is shown in text figure 31. In the control plants normally only one bud opens at a time on each cane. The rose flowers grown with additional light at 68° F. in 1927 shown in figure 31 were of better keeping quality than those grown at 78° in the same year but it required longer for them to develop. They still retained at the low temperature the characteristic of opening two or three flowers in a cluster at one time which was found to be true at the high temperature when grown with both additional light and carbon dioxide.

Nasturtium flowered profusely in Greenhouse 2 with additional light and carbon dioxide. Additional light alone was little better than the control conditions in forcing flowers. The great amount of flowering in House 2 is shown in text figure 32. The plants are all 69 days old from seed. The first flowers appeared in House 2 when the plants were 38 days old and in both House 1 and the control at 52 days of age. In the final yield of flowers during the experiments House 2 was first, House 1 second, and the control lowest. Nasturtium plants grew more rapidly at the higher temperature in 1925 and flowered earlier. In the low temperature series of 1926 House 2 plants flowered first at the age of 54 days and House 1 and control plants one week later.

Eggplants with additional light and gas (House 2) grew very rapidly and set several large fruits. Plants grown in House 1 were second in amount of fruit set. The controls did not fruit during the experiment. Text figure 33 shows the plants grown under the different conditions. The plants were in the conditions 67 days when the photograph was taken. All were small plants about three inches high when the experiments were started on February 28.

Tomato has been mentioned as an outstanding example of a plant which does not withstand continuous artificial illumination. Geranium and coleus also fall naturally into this group, the only difference being in the degree of injury. Photographs of these two species are shown in text figures 33 and 34. The injury of continuous illumination is apparent in both cases. These two species always managed to survive during the period of the experiments but they were always reduced to a stem with only a few small leaves remaining alive. Geranium, in contrast to tomato,



TEXT FIG. 31. Premier and Hoosier Beauty roses grown in gantry crane house with additional light and carbon dioxide showing the effect in forcing clusters of three sturdy roses at one time. New canes with flowers were produced from the root-stock in about the same time required to produce a single flower bud from existing canes in the control plants. TEXT FIG. 32. Nasturtium plants 70 days old from seed. The one in the center receiving both additional light and gas is flowering profusely. The plants from Greenhouse 1 receiving additional light only are little better than the controls (at right) on the normal length of day. TEXT FIG. 33. Geranium showing the flowering with supplementary light in Greenhouses 1 and 2 and the injury of continuous illumination (24 hour day). The control plant is at the right. TEXT FIG. 34. Variegated coleus, showing the increased growth with both additional light and gas (Greenhouse 2) as compared with additional light only (Greenhouse 1) at left and control greenhouse at right. The 24 hour day plant shows considerable light injury. TEXT FIG. 35. Eggplant showing the additional growth and fruiting with additional light only (Greenhouse 1) and with both light and gas (Greenhouse 2 center). The control at right did not fruit during the experiments.

grew well with continuous illumination in the gantry crane house in the 1927 experiments with 12 hours of daylight in combination with 12 of artificial light. This may be due to the difference in quality of the light source, sunlight always being more favorable for plant growth than that of the filament lamp. It may also be due to short periods of recovery at low intensities of daylight in the late afternoon or on cloudy days in February when the artificial light source was off, while those under continuous artificial illumination had practically no rest period or appreciable decrease in intensity during the entire period of the experiment.

#### PRACTICAL APPLICATION

These experiments were undertaken to determine some of the effects of environmental factors on plant growth rather than the possible application of such information as might be obtained to the commercial growing of plants in greenhouses. However, it is believed that some items of cost should be included in this report for the information of those interested in reproducing conditions similar to those already outlined for growing plants. 124 jars of the two gallon size were filled with soil and placed in the constant-light room for each experiment. One or more plants, depending upon size, were grown in each jar. Assuming that each jar contained only one plant 124 plants can be grown on a 24 hour day during each experiment with artificial light only. The cost for refrigeration, steam, and operation of four motors together with the attendants amounted to about \$26.00 per 24 hour day. The cost for lighting current at four cents per kilowatt hour was \$36.00 per day or about 29 cents per jar of plants. The total cost of operation therefore was \$62.00 per day. The 1925 experiments were continued for 75 days making a total cost of \$4650.00 or about \$37.00 per jar of plants. The total cost of lighting current alone was \$21.00 per jar. The additional item of carbon dioxide from tanks cost about \$8.00 per day and is not included in these calculations. The calculations also do not include the original cost of machinery and equipment, or depreciation. Many plants were grown throughout their life history in less time than 75 days. It has already been pointed out that many plants produce very little additional growth on a 24 hour day as compared to an 18 hour day, and also that some plants such as the tomato grow better at a lower light intensity. The cost calculations above could be reduced considerably therefore if a practical application were the aim.

It is believed that some application will be found, however, in supplementing daylight with artificial light for a period of three to six hours each night rather than in the use of artificial light entirely. In the case of the gantry crane the cost per day for six hours of supplementary lighting was \$11.52. 130 jars of plants were grown at a cost of about nine cents per day per jar or at a total cost of \$6.75 for the entire 75 days. A number of plants could be brought into full production with the intensities used

in the gantry crane houses during these experiments in 40 days, and since electric power could be used during the early morning hours when there is a small load on the lines it could be obtained for at least half of the price per kilowatt indicated above. The estimated cost would then reduce to about \$1.80 per jar for the entire growth period. This does not include the cost of lamps, equipment, or carbon dioxide.

#### DISCUSSION AND SUMMARY

This report is concerned with the growth of plants in artificial climates. Some of the plants were grown with artificial light only as a source of energy for photosynthesis. Other plants were grown with daylight supplemented with artificial light for six to 12 hours each night. An attempt was made to grow plants throughout their life history with photosynthesis at or near its maximum rate by supplying a high light intensity and long day along with increased carbon dioxide concentration and a relatively high temperature. The effect of length of day on certain species was also studied in various combinations of temperature and carbon dioxide supply. Chemical analyses of many plants grown under the different conditions are given, together with a discussion of the effect of various factors on the percentage carbohydrate and nitrogen in various tissues.

There is a difference in the percentage carbohydrate in tomato leaves, depending on the time of day at which they are sampled. When allowed to remain in darkness for 17 hours after exposure the total carbohydrate and especially the sucrose and dextrose fractions decrease considerably. After 40 hours in darkness these fractions decrease to approximately one-third of the original value. Depending upon when the plants are sampled, in relation to their light exposure period, various values for the carbohydrate-nitrogen ratio may be obtained. This variation is due to changes in carbohydrate since total nitrogen remains practically the same.

No relation was found between carbohydrate and nitrogen content and flowering in either long day plants such as radish and lettuce, or in *salvia*, a short day plant, or buckwheat, an everblooming type. It was found that the percentages of carbohydrate and nitrogen in general could be changed by varying light intensity, length of day, or in some plants by changing the nutrient supply when the plants were grown in sand instead of soil. The range of variation of these fractions depends upon the plant species. For *salvia* the range of total carbohydrate on a dry weight basis is narrow, since even the five hour day plants are able to maintain a high level of total carbohydrates. Total nitrogen also was restricted to a comparatively narrow range from 5 to 19 or 24 hour days. The application to the soil of large quantities of nitrate made practically no difference in the total nitrogen content of the aerial portion of *salvia* plants although it resulted in considerable foliar injury. This plant is apparently able to hold both the carbohydrate and nitrogen fractions within a narrow range when grown



under conditions which greatly affect the range in lettuce, radish and buckwheat. *Salvia* plants were kept from flowering by illumination for six hours each night from January to December with very little change in either the carbohydrate or nitrogen fractions.

The tomato was found to be the most light sensitive of any plant grown in these experiments. It will not withstand a 24 hour day at an intensity which causes little or no injury to other plants. The plants set fruit on all day lengths from seven to 19 hours but did not fruit on either five or 24 hour days. Fruit production and weight per plant were maximum in rapidly growing high carbohydrate, low nitrogen plants grown with daylight plus six hours of artificial light each night and with increased carbon dioxide supply. At higher intensities day lengths of 17 and 19 hours are injurious. When the plants are receiving 12 hours of artificial light at night more than six hours of sunlight is injurious. In general carbohydrates and weight per plant increase with day length up to the point where foliar injury begins to be effective in holding the plants back. At a low light intensity this increase holds up to a 17 or 19 hour day, while at higher light intensities the peak is reached on a 12 hour day. On long days total nitrogen was decreased to less than one percent of the dry weight. On a percentage of dry weight basis the ratio of total carbohydrate to total nitrogen closely parallels total carbohydrate. It is seen, therefore, that there is little relation between this ratio and the setting of fruit.

It is thought that the long day injury to tomato plants is produced by a breaking down in the process of photosynthesis rather than by too great an accumulation of the products of the process, since the injury can be produced with a low light intensity which results in no accumulation of carbohydrates in the leaves as compared to greenhouse plants.

Cabbage plants were found to increase in weight of tissue produced and in total carbohydrate with length of day up to 17 or 19 hours followed by a decrease on continuous illumination. The percentage total carbohydrate was usually doubled on a 19 hour day as compared to a five hour day. On long days this plant produced over 50 percent of the dry weight in easily available carbohydrates. Total nitrogen decreased with day length to 19 hours and increased slightly on a 24 hour day.

Red clover grew and flowered especially well with daylight supplemented by six hours of artificial light from the gantry crane in the greenhouses where carbon dioxide concentration was increased. Plants were grown from seed to flower in 38 days during the month of March and the first week in April while the control plants did not flower during the experiments. Carbohydrates increased and nitrogen decreased with increasing day length and increased carbon dioxide supply.

Four varieties of soy beans, Mandarin, Peking, Tokio and Biloxi, which Garner and Allard had observed to flower in June, July, August, and September respectively, were found to flower in the control greenhouse.

Of these only Mandarin and Biloxi flowered with additional light and carbon dioxid. Carbohydrates and weight per plant increased and nitrogen decreased with additional light and carbon dioxid.

Cucumber plants were found to absorb nitrate readily in contrast with salvia which absorbs little of this salt. This was especially true on the long days where a shortage of nitrogen had been produced due to an accumulation of carbohydrates. Total carbohydrates and weight per plant increased with additional light and higher concentrations of carbon dioxid.

Tuber production in potatoes, variety Irish Cobbler, was found to be favored by low temperature in combination with high light intensity and long days. High temperature (78° F.) produced weak stems with little or no tuberization, although weight of the aërial portion produced at high temperature was usually greater than at low temperature (68° F.). This variety formed many large tubers when grown with continuous illumination. The failure of previous workers to get tuberization on a long day is believed to be due to the high temperature conditions which were associated with long days in their experiments. Since high light intensity, long days, and cool air temperature greatly favor tuber production it is thought that these factors account for the high yields of potato tubers in high latitudes and higher altitudes.

Small grains such as barley and spring wheat, in contrast to potatoes, will grow well and yield well at a high temperature (78° F.) if given additional light and carbon dioxid. The production of these grains is not favored by low temperature when day length is long and carbon dioxid supply is abundant. The weight per plant of barley increases with day length up to a 19 hour day. Total carbohydrates also increase and nitrogen decreases. The feeding of nitrate was found to make little or no difference in the total percentage of nitrogen in the barley plant, the percentage remaining high only when carbohydrate synthesis was restricted by short days.

Winter wheat of the Turkey Red variety, did not head in these experiments, while Hybrid 128, a second variety of winter wheat, produced several heads on 18 and 24 hour days. Spring wheat, variety Blue Stem, headed especially well with additional light and carbon dioxid. This variety was grown from seed to head in 31 days.

Several ornamental plants such as roses, sweet peas, snapdragons, petunia, and nasturtium grew and flowered remarkably well with additional light and carbon dioxid. Both geranium and coleus were greatly injured, however, by continuous artificial illumination, the injury being similar to tomato but not quite as severe. In contrast to tomato these plants could be grown with little or no injury with continuous illumination if sunlight was used as a light source during the day and was supplemented with 12 hours of artificial light each night. Sunlight, in general, is a better light source for plant growth than the incandescent filament lamp.

A practical application of artificial light to the growing of plants will no doubt be found in supplementing daylight during the winter months with three to six hours of artificial light each night. The cost of growing plants with artificial light alone is prohibitive except for experimental or demonstrational purposes. In addition sunlight is a better source of energy for growing plants than artificial sources now available.

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## NAPHTHALENE FUMIGATION AT CONTROLLED CONCENTRATIONS<sup>1, 2</sup>

By ALBERT HARTZELL and FRANK WILCOXON

### ABSTRACT

A method of maintaining a constant concentration of naphthalene is described as an improvement on former methods of fumigation with this substance. A concentration of 0.008 lbs. of naphthalene per 1000 cu. ft. of air maintained for eight hours was found to kill red spider mite (*Tetranychus telarius*), cyclamen mite (*Tarsonemus pallidus*), the onion thrips (*Thrips tabaci*), and the black grain thrips (*Heliothrips, femoralis*) without injury to a number of plants that have proved intolerant to methods used previously.

### INTRODUCTION

With the increased use of naphthalene as a greenhouse fumigant for the control of mites and thrips the question of a suitable method of volatilizing this material has arisen. Among the methods employed are broadcasting along the borders (9), volatilization with a lamp (4, 6), and finally the substitution of an electric hot plate (5). The importance of maintaining a slow uniform rate of volatilization has been stressed by all investigators. Volatilization by means of lamps or electric hot plates has proved satisfactory for the more tolerant species and varieties, but certain plants are injured (5) by naphthalene vapor when these methods are used. The use of any method involving heat suffers under the disadvantage that a rather high concentration of naphthalene vapor is produced in the immediate neighborhood of the apparatus. This concentration will exceed the saturation value in parts of the greenhouse removed from the point of volatilization, and will cause deposition of naphthalene on the plants with consequent injury to those which are sensitive. If the naphthalene vapor could be introduced without heat, such a deposition would not occur, and injury would be reduced to a minimum. It would be necessary, of course, to supply the naphthalene vapor at a rate sufficient to compensate for leakage and to maintain the desired concentration for as many hours as were found needful for control. By passing a current of air over naphthalene at the same temperature as the greenhouse it should be possible to maintain almost any desired

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concentration up to that corresponding to the sublimation pressure of naphthalene, which would be the maximum attainable.

### THE APPARATUS

In order to meet the requirements as outlined above, a naphthalene saturator was constructed in the form of an air tight rectangular metal box, 47 in. long, 35 in. wide, and 35 in. high. This box (Fig. 59) was divided into four compartments by three vertical baffles, and each compartment contained eight horizontal shelves. Air was drawn in at one end of the box, and travelled through each compartment in succession and was expelled through an opening in the top of the box at the opposite end from the point of entry. A motor-driven blower mounted on the top of the box served to draw the air through the saturator and expel it into the fumigation chamber. The air in passing over the shelves, which were each filled with a single layer of naphthalene balls, became partially saturated with naphthalene vapor. The final concentration of naphthalene in the air at a given temperature could be controlled either by varying the number of shelves filled with naphthalene balls, or by varying the speed of the blower. In these experiments the speed of the blower was constant, 1144 R. P. M., and the first mentioned method of control was used. With 24 shelves filled (which required 79 lbs. of naphthalene), and with an air velocity of 52 cu. ft. per minute, the time of contact of the air with the naphthalene was approximately one minute and the area of contact was approximately  $12\frac{1}{3}$  sq. ft., assuming the naphthalene balls to be spheres of uniform size. The number of pounds of naphthalene balls required to give satisfactory control of mites and thrips without injury to the host plants was determined by trial. In general, satisfactory results were obtained with 79 lbs. When 64 lbs. were used the control of cyclamen mite was possible with a 12-hour fumigation, but rather unsatisfactory results were obtained with red spider mite. With 93 lbs. considerable injury to the plants was experienced.

A greenhouse compartment situated between two larger sections was used as a fumigation chamber (Plate 20, Fig. A). The capacity of this chamber was 850 cu. ft. The height and width of the compartment was the same as the adjacent greenhouse sections and differed only from them in being about one-seventh their length. As the compartment was a unit in the same greenhouse range, the conditions of temperature, humidity, and light intensity were comparable to those of the adjoining greenhouses.

The naphthalene saturator described above was installed in the chamber and naphthalene free air was drawn into the saturator from an adjacent greenhouse section by means of a duct. Upon completing its path the air laden with naphthalene vapor was deflected upward from the exhaust of the blower into the fumigation chamber. Opposite the saturator was a shelf built the same height as the greenhouse benches on which potted plants to be fumigated were placed. In very cold weather it was sometimes found that a small amount of naphthalene condensed on the panes of glass that constituted the roof of the chamber. When this happened it was desirable to remove the deposit since condensing moisture laden with naphthalene dropped on the plants and caused injury.

Fumigations were made both day and night. In this study special emphasis was laid on testing the tolerance of plants that previous investigation had shown to be sensitive to naphthalene vapor. These were carefully checked with plants of the same age that had not been fumigated in order to note any possible delayed effect that the fumigation might have on plant growth. Plants infested with mites and thrips were fumigated and the results compared with data on the natural mortality of these species. Daylight fumigations were begun at 10 A. M. and continued until 4 P. M., a period of six hours. If an eight-hour period was desired the fumigation was terminated at 6 P. M. Night fumigations were run for a period of 15 hours, beginning at 5 P. M. and continuing until 9 A. M. the following morning. The plants were watered before fumigation as previous experience had shown that plants fumigated under dry conditions were liable to be injured.

#### ANALYSIS OF GREENHOUSE AIR FOR NAPHTHALENE

In greenhouse fumigation experiments few attempts to determine the actual concentration of the active agent are on record. Eddy and Geddings (1) give data on the determination of hydrogen cyanide in a fumigation chamber. It was considered desirable to attempt to determine the naphthalene concentration in the greenhouse air during a fumigation, although it was known that this was very small. The method adopted was that of Gair (3). Air was drawn by a water pump through an absorption train consisting of two gas washing bottles each containing 175 cc. of acetic acid of sp. g. 1.044, followed by a third bottle containing 150 cc. of saturated picric acid solution. The air was finally passed through a small laboratory flow meter. At the conclusion of a run, the naphthalene was precipitated as the picrate by the addition of

500 cc. of saturated picric acid solution as described by Gair. The naphthalene picrate was filtered through a weighed Gooch crucible, dried in a desiccator and weighed. From the weight obtained, and the volume of air measured by the flow meter, the concentration of naphthalene vapor in the greenhouse air could be calculated. Owing to the small concentration, it was necessary to continue the run over several fumigation periods in order to obtain a weighable amount of naphthalene

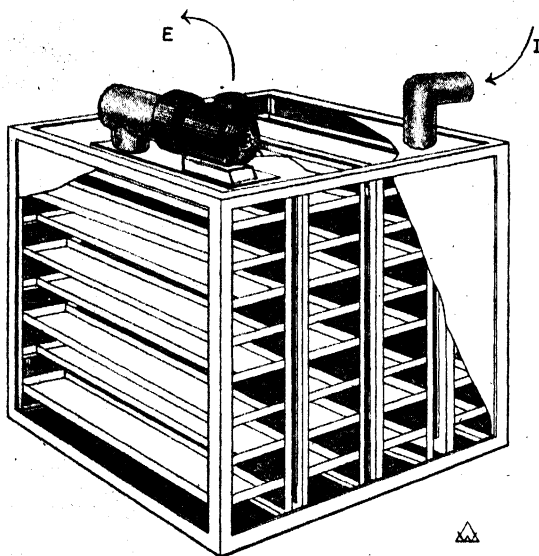


Fig. 59.—Naphthalene saturator consisting of a galvanized iron box containing 32 horizontal shelves separated by baffles. *I*. The naphthalene free air is drawn in at this point from an adjacent greenhouse section. *E*. The air laden with naphthalene is expelled at this point from the motor-driven blower after its passage over the shelves filled with a single layer of naphthalene balls.

picrate. The result thus obtained is an average value, and required four or five days for a single determination. One such experiment gave a concentration of naphthalene of 0.0076 lbs. per 1000 cu. ft., and a subsequent determination gave 0.0085 lbs. per 1000 cu. ft. The



average temperatures during these experiments were 86°F. and 89°F. respectively. A determination in which the sample was taken at the point of exit from the saturator gave a concentration of 0.0128 lbs. per 1000 cu. ft. Roark and Nelson (8) have published tables showing the amount of naphthalene vapor in saturated air at various temperatures. From the value for 86°F. which is given as 0.06 lbs. per 1000 cu. ft., it appears that in our experiments the air in the neighborhood of the plants was approximately 13% saturated with naphthalene vapor. This concentration if maintained for a sufficient length of time, gave satisfactory control of mites and thrips with less injury than previous methods had shown.

By weighing the charge of naphthalene before and after a series of fumigations it was found that the rate of loss per hour was 0.0884 lbs. This includes any loss that might take place between periods of fumigation.

#### PLANT TOLERANCE

It has been shown in previous publications (4, 5) that certain species and varieties of plants are injured by the lamp and hot plate methods of volatilization. This was found to be true even with fumigations made at night and with amounts not exceeding two ounces per 1000 cu. ft. of greenhouse space. Of 150 species and varieties tested by the above methods more or less foliage injury resulted to forty-two. A list of these intolerant plants follows:

<i>Capsicum annuum</i> var. <i>longum</i> (pepper)	<i>Nicotiana suaveolens</i>
<i>Fagopyrum esculentum</i> (buckwheat)	<i>Nicotiana sylvestris</i>
<i>Fuchsia speciosa</i>	<i>Nicotiana tabacum</i>
<i>Glycine max</i> (soy bean)	var. <i>gigantia</i>
<i>Linaria</i> sp.	var. <i>purpurea</i>
<i>Lycopersicum esculentum</i> (tomato)	<i>Nicotiana trigonophylla</i>
<i>Lythrum salicaria</i> var. <i>roseum</i>	<i>Nycterinia capensis</i>
<i>Magnolia</i> sp.	<i>Oxalis</i> sp.
<i>Martynia proboscidea</i>	<i>Papaver</i> sp. (poppy)
<i>Matricaria alba</i>	<i>Papaya</i> sp.
<i>Maurandia</i> sp.	<i>Pelargonium</i> sp. (geranium)
<i>Nemesia</i> sp.	<i>Plantago major</i> (broad leafed plantain)
<i>Nemophila</i> sp.	<i>Philadelphus</i> sp. (mock orange)
<i>Nicotiana langsdorffii</i>	<i>Physalis</i> sp.
<i>Nicotiana longiflora</i>	<i>Raphanus sativus</i> (radish)
<i>Nicotiana nudicaulis</i>	Rose var. <i>Button hole</i>
<i>Nicotiana paniculata</i>	<i>Ribes nigrum</i> (black currant)
<i>Nicotiana plumbaginifolia</i>	<i>Schizanthus wisetonensis</i>
<i>Nicotiana quadrivalvis</i>	<i>Trifolium pratense</i> (red clover)
<i>Nicotiana repanda</i>	<i>Tropaeolum majus</i> (nasturtium)
<i>Nicotiana rustica</i>	<i>Vitis vinifera</i> (grape)
<i>Nicotiana sanderae</i>	

While it was not found feasible to test every species and variety in the above list with fumigations at controlled concentrations, it was found possible to test the more common species grown in greenhouses and in addition a number of important plants not included in the above list. Reference to Table 1 will show that of a total of 32 species and varieties of plants fumigated, only three species were found to be intolerant, namely buckwheat (*Fagopyrum esculentum*), soy bean (*Glycine max*), and Tabasco pepper (*Capsicum annuum* var. *conoides*). The last named plant was only slightly injured, the very oldest leaves turning yellow and falling off. Other varieties of pepper were uninjured. Seedling buckwheat plants were found to be by far the most sensitive of the three (Plate 21, Figs. A and B). A comparison of the above list of intolerant plants with Table 1 shows that of a dozen species and varieties which were found to be intolerant to naphthalene fumigation by the lamp and hot plate methods, only two were severely injured by our improved method. Included in these tests were such important greenhouse plants as pepper (*Capsicum annuum*) (five varieties), tomato (*Lycopersicum esculentum*), *Oxalis* sp., geranium (*Pelargonium* sp.), radish (*Raphanus sativus*), rose seedlings and nasturtium (*Tropaeolum majus*), which could not be fumigated with safety by the older methods, but which were found to be tolerant when fumigated at a properly controlled concentration. Irish Cobbler, Green Mountain, and Bliss Triumph potatoes (*Solanum tuberosum*), egg-plant (*Solanum melongena* var. *esculentum*), turnip (*Brassica rapa*), *Bryophyllum* sp., *Calendula officinalis*, China aster (*Callistephus chinensis*), *Centaurea imperialis*, cucumber (*Cucumis sativus*), *Datura stramonium*, carrot (*Daucus carota* var. *sativa*), Sudan grass (*Holcus sudanensis*), Turkish tobacco (*Nicotiana tabacum*), bean (*Phaseolus vulgaris*), *Sedum* sp., white clover (*Trifolium repens*), and wheat (*Triticum aestivum*) were also found to be tolerant. Even cyclamen plants in flower were not injured (Plate 20, Figs. B and C). It should be noted that all these plants were fumigated in daylight at temperatures ranging from 72° F. to 100°F., during both cloudy and sunny weather and that no injury resulted even with concentrations slightly higher than that found necessary to kill mites and thrips. A careful comparison of fumigated plants with their corresponding check plants which were not fumigated, showed no evidence of stunting or of delayed injury.

The writers' previous experience had been unfavorable with daylight fumigations in bright sunlight and with the higher range of temperatures. Apparently the slower uniform rate of volatilization of naphthalene by

this method results in a concentration considerably below the tolerance limit to foliage, which is in direct contrast to the uneven rate obtained by means of the hot plate and lamp methods with the resultant injury to foliage. The chief disadvantage of daylight fumigation is the difficulty of keeping the temperature from rising too high; this especially is true with plants such as cyclamen and potato which are normally grown at lower temperatures. It is interesting to note in this connection that a concentration which would injure young buckwheat plants but which would not injure six-inch tomato plants (var. Bonny Best) was found to be suitable for all species and varieties as shown in Table 1, with the exception of buckwheat, soy bean, and Tabasco pepper.

TABLE 1. EFFECT OF NAPHTHALENE FUMIGATION AT CONTROLLED CONCENTRATION ON HOST PLANTS

Name of Plant	Number of Plants	Height of Plants Inches	Daylight Fumigation 6 hours	
			Temperature °F.	Relative Humidity Percent
<i>Brassica rapa</i> (turnip).....	40	4	82-100	70
<i>Bryophyllum</i> sp.....	5	6	82-100	70
<i>Calendula officinalis</i> .....	5	6	78-90	67
<i>Callistephus chinensis</i> (China aster).....	24	4	68-98	68
<i>Capsicum annuum</i> var. <i>abbreviatum</i> .....	36	12	82-100	70
var. <i>acuminatum</i> .....	36	10	82-100	70
var. <i>cerasiforme</i> (red cherry pepper).....	36	8	82-100	70
var. <i>conoides</i> (Tabasco pepper)*.....	36	15	82-100	70
var. <i>fasciculatum</i> (Red Japan cluster pepper)	36	12	82-100	70
var. <i>grossum</i> (bell pepper)	36	12	82-100	70
<i>Centaurea imperialis</i> .....	5	6	78-90	67
<i>Cucumis sativus</i> (cucumber).....	10	4	86-100	48
<i>Cyclamen indicum</i> .....	15	10	80-100	50
<i>Datura stramonium</i> .....	30	12	80-100	78
<i>Daucus carota</i> var. <i>sativa</i> (carrot).....	50	3	68-98	68
<i>Fagopyrum esculentum</i> (buckwheat)*.....	200	10	82-100	70
<i>Fuchsia speciosa</i> .....	5	15	78-90	67
<i>Glycine max</i> (soy bean)*.....	200	6	80-100	78
<i>Holcus sudanensis</i> (Sudan grass).....	100	12	68-98	68
<i>Lycopersicum esculentum</i> (tomato).....	50	6	76-86	68
<i>Nicotiana tabacum</i> .....	24	—	82-100	70
<i>Oxalis</i> sp.....	500	—	82-100	70
<i>Pelargonium</i> sp. (geranium).....	5	12	78-90	67
<i>Phaseolus vulgaris</i> (bean).....	10	—	72—	58
<i>Physalis francheti</i> .....	10	4	82-100	70
<i>Prunus persica</i> (peach).....	10	24	80-84	56
<i>Raphanus sativus</i> (radish).....	50	4	82-100	70
Rose seedlings.....	20	6	81-95	64
<i>Sedum</i> sp.....	5	6	76-96	50
<i>Solanum melongena</i> var. <i>esculentum</i> (egg-plant).....	10	—	68-98	60
<i>Solanum tuberosum</i> var. Irish Cobbler potato.....	12	—	78-100	56

TABLE 1—Continued

<i>Solanum tuberosum</i> var. Bliss Triumph...	40	—	78-100	56
<i>Trifolium pratense</i> (red clover).....	200	8	82-100	70
<i>Trifolium repens</i> (white clover).....	200	4	82-100	70
<i>Triticum aestivum</i> (wheat).....	100	4	80-100	50
<i>Tropaeolum majus</i> (nasturtium).....	50	8	82-100	70
Night fumigation 15 hours				
<i>Capsicum annum</i> var. abbreviatus.....	36	6	72-80	78
var. acuminatum.....	36	6	72-80	78
var. cerasiforme (red cherry pepper).....	36	6	72-80	78
var. conoides (Tabasco pepper)*.....	36	6	72-80	78
var. fasciculatum (Red Japan cluster pepper)	36	6	72-80	78
var. grossum (bell pep- per).....	36	6	72-80	78
<i>Glycine max</i> (soy bean)*.....	100	4	76-86	68
<i>Lycopersicum esculentum</i> (tomato).....	24	18	72-80	78
<i>Nicotiana tabacum</i> (tobacco var. Turkish).	24	3	72-86	68
<i>Oxalis</i> sp.....	200	—	72-80	78
<i>Trifolium pratense</i> (red clover).....	200	—	72-80	68

\*Foliage injured.

## CONTROL OF GREENHOUSE PESTS

It was found during the course of this investigation that naphthalene fumigation would control cyclamen mite (*Tarsonemus pallidus*) on various greenhouse plants such as cyclamen and pepper. The minimum period of exposure necessary to obtain satisfactory control was found to be six hours when 79 lbs. of naphthalene was used in the saturator. It has been shown elsewhere (3) that the red spider mite<sup>1</sup> (*Tetranychus*

TABLE 2. PERCENTAGE CONTROL OF GREENHOUSE PESTS TO NAPHTHALENE VAPOR

Expo- sure to naphtha- lene vapor Hours	Temperature		Rela- tive humid- ity Per cent	Red Spider mite		Cyclamen mite		Thrips tabaci		Thrips femoralis	
	Maxi- mum °F.	Mini- mum °F.		Num- ber of speci- mens	Per cent control	Num- ber of speci- mens	Per cent control	Num- ber of speci- mens	Per cent control	Num- ber of speci- mens	Per cent control
	°F.	°F.									
6	84	80	56	—	—	500	100	200	100	500	96.1
6	100	78	46	171	74	500	100	—	—	—	—
8	100	80	50	187	94.1	241	100	—	—	—	—
8	100	80	63	267	99	500	98.6	—	—	—	—
8	100	78	70	525	97.3	—	—	—	—	—	—
8	75	74	72	393	94	500	99	502	99	500	99

*telarius*), the onion thrips (*Thrips tabaci*) and the black grain thrips (*Heliothrips femoralis*) are controlled by naphthalene fumigation. As indicated in Table 2, it required a minimum of eight hours to obtain a

<sup>1</sup>The term red spider mite is used in preference to the term red spider, following the suggestion of the Committee on Nomenclature of the American Association of Economic Entomologists.

satisfactory control of thrips and of the red spider mite. Thus it follows that the three species could be controlled by eight-hour fumigations and if the plants were infested with the cyclamen mite alone, a six-hour fumigation was sufficient or the amount of naphthalene could be reduced to 64 lbs. and the period lengthened to 12 hours.

The question of the resistance of the various stages of the red spider mite to naphthalene vapor has arisen. Read (8) has shown by laboratory experiments that it required at least eight hours' exposure of the eggs to a saturated atmosphere of naphthalene to prevent hatching; at 60°F. they were not killed when exposed for a period of 30 hours. To determine the effect of this method of fumigation on hatching of eggs, leaves from plants fumigated for eight hours at an average temperature of 86°F. were placed in petri dishes and counts of larvae (first stage) made at various intervals of time. A similar series of leaves from unfumigated plants served as a check. Table 3 shows the number of larvae on these leaves in the control and fumigated series. The temperature during this period was 71°F. It will be observed that fumigation by this method has had a considerable effect on the hatching of the eggs. In the control 21% of the eggs had hatched after 120 hours while in the case of the fumigated series only about 3.5% had hatched in this time.

TABLE 3. EFFECT OF NAPHTHALENE FUMIGATION ON HATCHING OF EGGS OF RED SPIDER MITE

Hours After Fumigation	Fumigated Number of eggs	Number of Larvae (First Stage)	Control Number of Eggs	Number of Larvae (First Stage)
24	964	11	394	16
48	—	20	—	20
72	—	30	—	72
120	—	34	—	84

A study of the comparative resistance of the larva, protonymph, deutonymph, and the adult female to naphthalene vapor showed that there was a slight increase in resistance on passing from the larva to the adult and, furthermore, that the last two stages exhibit a significant difference in resistance when compared with the first two stages. When the  $X^2$  test for homogeneity (2) was applied to the data on the first and second stages grouped together as compared with the third and adult stages grouped similarly, the difference was found to be significant with odds greater than 100 to 1. The total number of individuals used in this comparison was 4204.

The possibility of recovery of the red spider mite after fumigation was considered. To test this point leaves from fumigated plants were kept in petri dishes under observation for a period of 120 hours and counts of

living individuals exclusive of the eggs and first stages, were made. Out of a total of 380 individuals 15 were alive 24 hours after fumigation and 11, 120 hours after fumigation. It appears, therefore, that the percent recovery in the 120 hours following an eight-hour fumigation was very low, while the mortality in the check was less than two percent.

### DISCUSSION

In the case of a toxic agent, where the concentration necessary to kill is not far removed from that which will injure the host plant, it is desirable to use the material at a constant concentration. If the concentration fluctuates widely during the experiment, injury may be experienced, even though control is incomplete. Naphthalene as a fumigant seems to be a case of this kind. The method of fumigation described in this paper provides an almost automatic control of the actual concentration, and also permits an experimental determination of the best time and concentration to use for a given purpose. The fact that by this method it was possible to fumigate plants that had previously proved intolerant to naphthalene, suggests that a more careful study of compounds already in use may be as valuable as a search for new toxic agents hitherto untried. The writers' experience with naphthalene as a greenhouse fumigant as applied by the hot plate and lamp methods has not been entirely satisfactory with mixed plantings. The difficulty of removing intolerant plants prior to fumigation greatly limits its use. The method described above has not yet been tested for large scale use but the principle involved, i.e., the use of a constant concentration of naphthalene throughout the fumigation period appears to be a step in the right direction.

### SUMMARY

A method of fumigating with naphthalene is described which permits a constant concentration of naphthalene to be maintained in the fumigation chamber throughout the experiment.

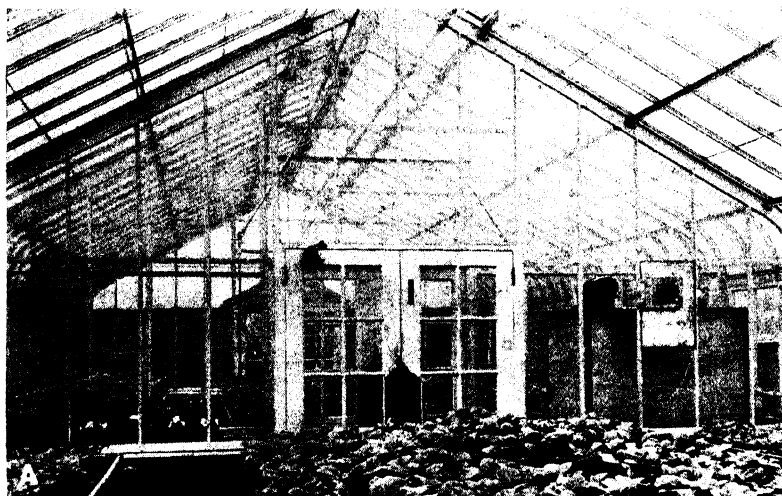
It has been established that a concentration of naphthalene 0.008 lbs. per 1000 cu. ft. if maintained for eight hours at an average temperature of 87°F. and an average relative humidity of 60% will give satisfactory control of the red spider mite, cyclamen mite, and thrips.

No injury was observed in these experiments to any plant except buckwheat, soy bean, and one variety of pepper. Fumigation was carried out in the daytime as readily as at night by this method.

A series of plants that had proved intolerant to naphthalene fumigation by previous methods was successfully fumigated by the method described.

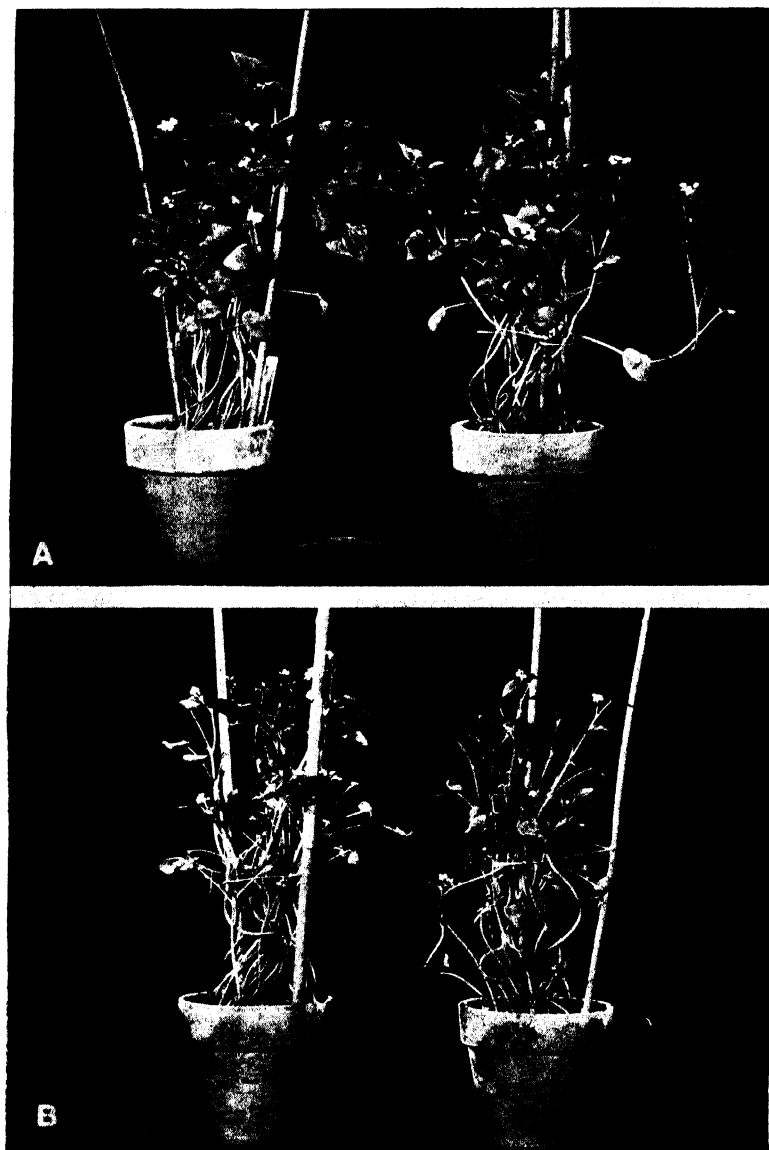
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- A. Fumigation chamber situated between two greenhouse sections with naphthalene saturator in place. Note the plants to the left to be fumigated.
- B. Cyclamen plants in flower that have been fumigated with naphthalene showing no injury to flowers or foliage.
- C. Cyclamen plants that were badly infested with cyclamen mite that have been fumigated with naphthalene with no foliage injury.





A. Buckwheat plants unfumigated.  
B. Buckwheat plants that have been fumigated with naphthalene showing foliage injury. Buckwheat is very intolerant to naphthalene fumigation and was found to be one of a few plants unsuitable for fumigation with naphthalene.

## SHORTENING THE REST PERIOD OF GLADIOLUS BY TREATMENT WITH CHEMICALS<sup>1, 2</sup>

F. E. DENNY

### INTRODUCTION

It is well known that freshly-harvested gladiolus corms, if again planted, do not germinate at once, even if the conditions that are ordinarily favorable for growth are present. The length of this rest period varies with different varieties but is usually two or three months.

On account of the response of such dormant plants as potatoes, lilac, apple, etc. to vapors of ethylene chlorhydrin, as reported in previous papers (1, 6), it seemed of interest to determine whether the dormancy of gladiolus corms could be broken in like manner, and if so under what conditions this result could be obtained, and whether the subsequent growth of the plants would be favorable for an early development of blooms.

Preliminary experiments indicated that the type of response resulting from the treatment depended upon the variety, and especially upon the stage of the rest period at which the treatment was applied. It was not until the late summer and autumn of 1929 that a supply of freshly-harvested bulbs of dependable quality, including several standard varieties, was available. This is a report of experiments in which ethylene chlorhydrin was used mainly, but in which comparisons were made also with certain other methods that have been suggested for shortening the rest period of gladiolus.

### VARIETIES USED

Corms of the no. 1 size of Halley, Alice Tiplady, Souvenir, Maiden's Blush, Mrs. Francis King, and Remembrance were obtained from Mr. A. Ludecke of Castle Hayne, North Carolina. Halley was harvested August 6, and the others on August 26, 1929. The corms were shipped to Yonkers by express, and the series of treatments began on August 10 for Halley and on September 2 for the other varieties. Tests of the different varieties were made at subsequent intervals in order to note the response at different stages of the rest period.

The corms that were not treated remained dormant for about two months, with the exception of Alice Tiplady, which after three months was still partly dormant and responded to treatment.

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> Herman Frasch Foundation for Research in Agricultural Chemistry, Paper no. 5.

## METHODS OF TREATMENT

*Ethylene Chlorhydrin.*—The bulbs were first peeled and then placed in glass jars of a capacity of  $3\frac{1}{2}$  liters, either museum jars with a clamp top, or battery jars with a saucer top which could be sealed tight with artists' molding clay. Before the jar was closed the required amount of 40 percent ethylene chlorhydrin was dropped from a pipette on a piece of cheese-cloth which was then spread loosely at the top of the jar. During the period of treatment the ethylene chlorhydrin evaporated and produced within the container a vapor the concentration of which varied with the amount of chemical used. The amounts per liter of air space within the container were varied from about 4 cc. per liter for four days to 1 cc. per liter for one day.

*Ethylene.*—The vessels for treating corms with ethylene were cans of either 17.5 or 35 liters capacity into which a measured amount of the gas was introduced. Molding clay was used for making a tight seal. The amount of ethylene used was one part ethylene per 1,000 parts of air within the container, and the length of exposure was usually six days, but in some cases three-day and twelve-day treatments were tried. Each second day the vessel was given a new charge of gas after thorough aëration. Harvey (2) used ethylene in the treatment of gladiolus at the rate of 1 : 1000 and the period of treatment was six days.

*Ether.*—The procedure with ethyl ether was the same as that for ethylene chlorhydrin. Only one concentration was tested, one cc. per liter, and the treatment lasted six days. Johannsen (3, p. 26) had previously used ether with bulbs without a satisfactory practical result, but Harvey (2) found the ether treatment to be successful with gladiolus. He used one-half teaspoonful of ether per 100 cubic inches for six days.

*Warm Temperature Storage.*—Some tests of this method of treatment were included because of the favorable results obtained by Loomis and Evans (4). Storage at 30° C. for three weeks was used in all of the tests, and in some of them additional procedures such as 30° for two weeks, 30° for four weeks, and 38° for one week were included. In some of the tests the corms were held dry in cloth bags during the storage period, and in others they were packed loosely in moist sphagnum moss.

## PLANTING

The lots contained 12 to 50 corms each, this being a sufficient number for a test when gladiolus corms are freshly harvested, since the dormancy is so pronounced that few, if any, of the untreated corms will sprout. Later in the season when they are coming out of the dormant period, larger numbers would be needed in order to take the variability of the sample into account. But the experimental work in this case was stopped when the untreated lots of a given variety showed even a few sprouts on planting.

After treatment the corms were planted in flats in moist soil and were

stored at room temperature until sprouting began. The flats were then placed in a greenhouse at a temperature of about 70° F. There were 12 to 16 corms in each flat.

### RESULTS

Since the effects of the treatments have depended so largely upon the stage of dormancy at which the treatments were applied, the results will be described first for the treatments applied at once after harvest, and then for the treatments applied at later stages. Also, because of the difference in the behavior of the various varieties it will be necessary to consider each variety separately.

#### Treatments Applied at Once After Harvest

*Souvenir*.—Of all varieties tested *Souvenir* responded in the most satisfactory manner. The results with this variety when bulbs harvested August 26 were treated September 2 are shown in text figure 1. The germination of the ethylene chlorhydrin lots was prompt, and the range of concentration giving good sprouting was from four cc. per liter for four days to two cc. per liter for two days. The germination was hastened even at one cc. per liter for one day, but some of the corms failed to respond.

Of the other methods tested, ether and ethylene failed to induce germination, and the warm-storage treatment, although having a detectable effect, was much behind even the poorest of the ethylene chlorhydrin lots.

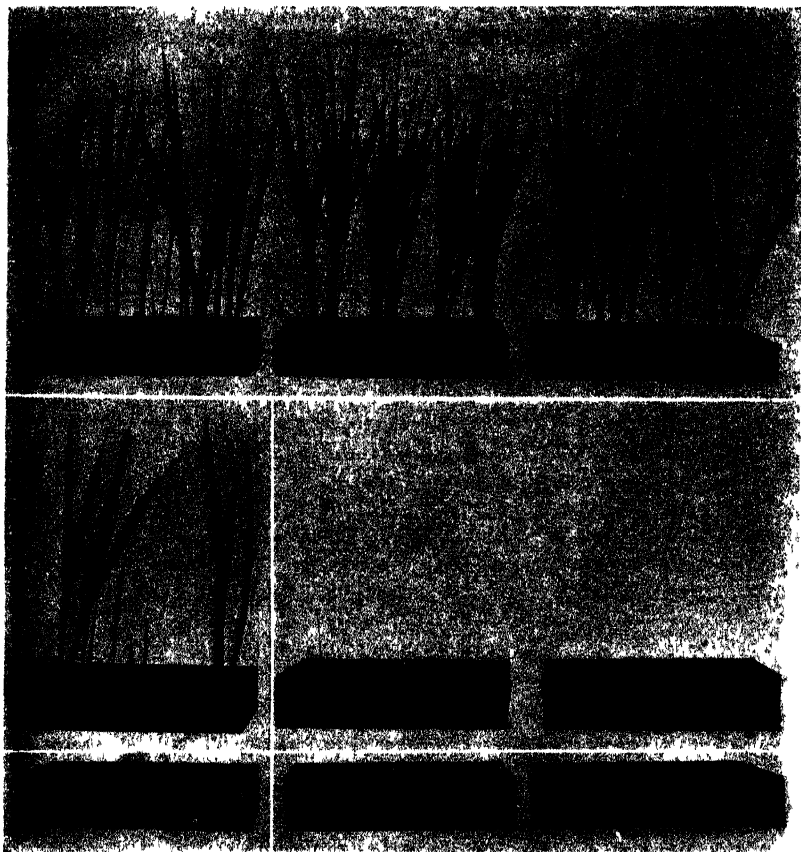
*Alice Tiplady*.—This variety was the most dormant of those tested in this experiment and yet it responded fairly well even at this early stage of dormancy to treatment with ethylene chlorhydrin. The concentrations, four cc. per liter for four days and three cc. per liter for three days, gave a definite hastening of development; but two cc. per liter for two days was much less effective and one cc. for one day failed completely. The ethylene, ether, and warm-temperature storage treatments were not successful in forcing germination of this variety at this stage of dormancy.

*Maiden's Blush*.—Concentrations ranging from four cc. per liter for four days to one cc. per liter for one day gave successful forcing of bulbs of this variety at this stage. The growth of the plants, however, was not so vigorous as that for *Souvenir* and *Alice Tiplady*. Sprouting was not induced by the other treatments tested.

*Mrs. Francis King*.—None of the treatments was successful with this variety at this stage. The ethylene chlorhydrin treatments were the only ones to induce sprouting, but the germination was poor on account of the large amount of rot which resulted. Even the untreated lot, however, showed considerable rot. Further experiments will be needed before a definite statement can be made regarding the application of the treatment to this variety.

*Remembrance*.—Of the varieties tested, this one offered the most stubborn resistance to treatments. The sprouting was not measurably hastened at

this early stage by any treatment tried, and even at later stages the result was never good up to the time at which the corms had gone through their natural dormant period, and had begun to sprout of their own accord. It is hoped to continue the experiments with this variety to determine whether the same behavior will be exhibited by crops of other years.



TEXT FIG. 1. Results of treatment of corms of gladiolus, variety Souvenir. Corms harvested August 26, treated September 2, this photograph made November 15. Top row, left to right: ethylene chlorhydrin, four cc. per liter for four days; three cc. per liter for three days; two cc. per liter for two days. Middle row: ethylene chlorhydrin, one cc. per liter for one day; storage at 30° C. for three weeks; ether, 1 teaspoon in 3.5 liter space for six days. Bottom row: ethylene 1 : 1000 for six days; check in closed container with air for four days; check in closed container with air for one day.

*Halley.*—Bulbs of this variety harvested August 6 and treated August 10 with ethylene chlorhydrin using four cc. per liter for four days, three cc.

per liter for three days, and two cc. per liter for two days when examined August 28 showed many sprouts about one-half inch long. But only in the four cc. per liter lot did these sprouts appear above ground, and in this case only 22 out of 36 produced plants of full height. The rest of the bulbs merely formed secondary corms as shown in text figure 2. Apparently the



TEXT FIG. 2. Top row left showing the formation of secondary corms as a result of treatment with vapors of ethylene chlorhydrin. Bottom row left, check lot not treated. At right, closer view showing secondary corms. *Gladiolus*, variety Halley. Harvested August 6, treated August 10, this photograph made November 7.

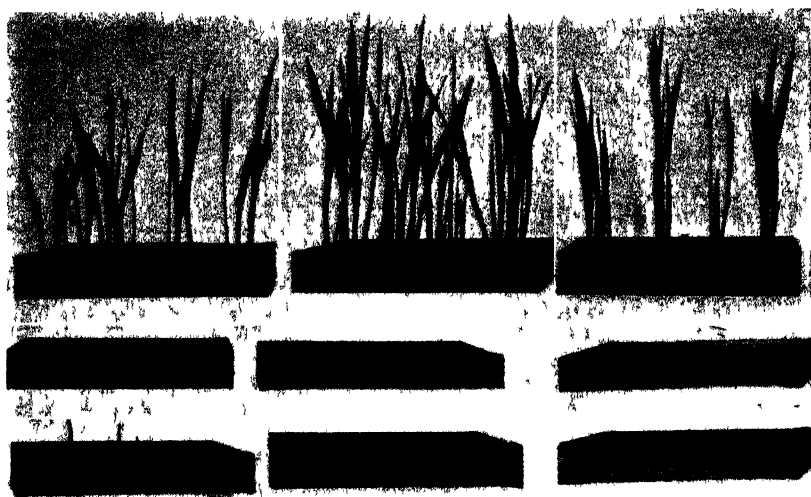
sprouts went back into dormancy again and instead of forming a shoot formed a new corm. A few of these small corms sprouted subsequently but most of them remained in the flats for several weeks in a fully dormant condition. The ethylene chlorhydrin treatments, therefore, were not completely effective when applied to Halley at this early stage. The other treatments (ethylene 1 : 1000 for six days, ether for six days, storage at 38° C. for one week, storage at 30° C. for three weeks) were not effective in inducing sprouting, nor were secondary corms formed in these cases.

#### Treatments Applied at Later Stages of the Rest Period

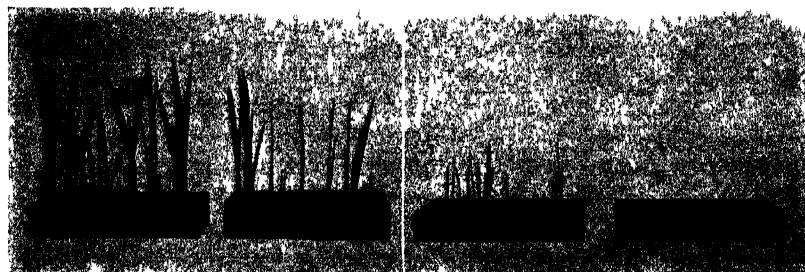
*Halley*.—Although, as stated in the preceding paragraph, treating Halley corms as soon as harvested did not induce satisfactory sprouting, the situation was entirely different when the treatments were applied at a later period. Thus, when corms harvested August 6 were treated September 5 the results shown in text figure 3 were obtained. Good forcing of growth resulted from various treatments with ethylene chlorhydrin, *e.g.*, four cc. per liter for one day, two cc. per liter for four days, two cc. for two days, one cc. for four days, one cc. for one day, although the last named was less effective than the others. A concentration of four cc. per liter for four days was too strong at this stage of the rest period and induced much rotting of corms. Among the other treatments tested it will be noted that only the ethylene treatment, 1 : 1000 for 12 days, hastened sprouting. The warm-temperature storage at 30° for two, three, or four weeks failed to break the dormancy of corms of this variety at this stage.

*Alice Tiplady*.—The result of the October 1st test with this variety is shown in text figure 4. Ethylene chlorhydrin, three cc. for two days, one cc.

for six days, one cc. for two days, all forced germination much ahead of the check. Warm storage  $30^{\circ}$  for three weeks also gave much quicker



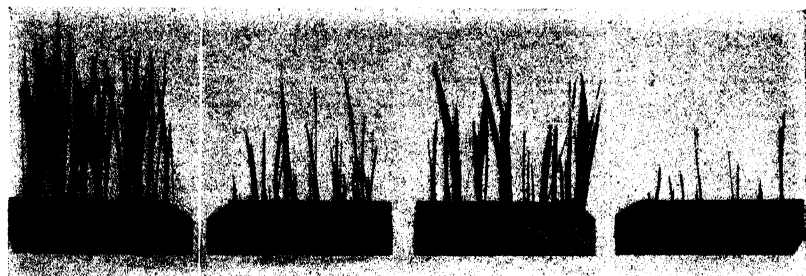
TEXT FIG. 3. Results of treatment of gladiolus corms, variety Halley. Corms harvested August 6, treated September 5, this photograph made November 12. Top row, left to right: ethylene chlorhydrin, four cc per liter for one day, one cc. per liter for one day; one cc. per liter for four days. Middle row: storage at  $30^{\circ}$  C. for two weeks, storage at  $30^{\circ}$  C. for three weeks; storage at  $30^{\circ}$  C. for four weeks. Bottom row: ethylene 1 : 1000 for 12 days, check in closed container with air for four days; check in closed container with air for one day.



TEXT FIG. 4. Treatment of gladiolus variety Alice Tiplady. Corms harvested August 26, treated October 1, this photograph made November 30. Left to right: ethylene chlorhydrin, three cc. per liter for two days; one cc. per liter for six days; storage at  $30^{\circ}$  C. for three weeks; check, not treated.

germination as shown in the third flat from the left in text figure 4. Later in the rest period when the untreated bulbs could germinate fairly well

without any treatment, the gain due to treatment was still distinct as seen in text figure 5. Ethylene 1 : 1000 for six days and warm-temperature



TEXT FIG. 5. Treatment of gladiolus variety Alice Tiplady. Corms harvested August 26, treated October 25, this photograph made December 30. Left to right: ethylene chlorhydrin, three cc. per liter for three days; storage at 30° C. for three weeks; ethylene 1 : 1000 for six days; check, in closed container with air for six days.

storage 30° for three weeks both showed good gains over the check lot, and were nearly as good as the ethylene chlorhydrin treatments.

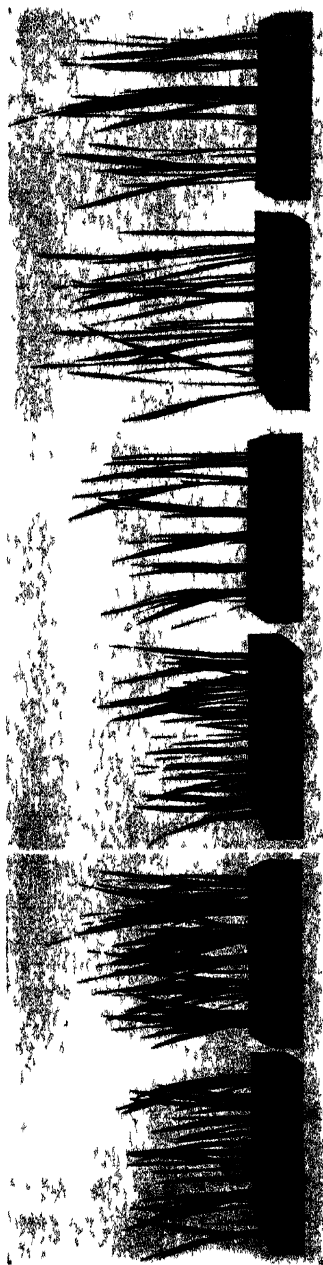
#### Comparison of Different Methods of Treatment

The ethylene chlorhydrin treatment gave better results in these tests than the other methods of treatment; but it must be remembered that the chlorhydrin method was tested over a wider range of concentration and period of treatment than any of the other methods. Hence, the results cannot be conclusive as a comparison of methods, since it is possible that the other methods could give equally good or better results if a more suitable combination of conditions could be found.

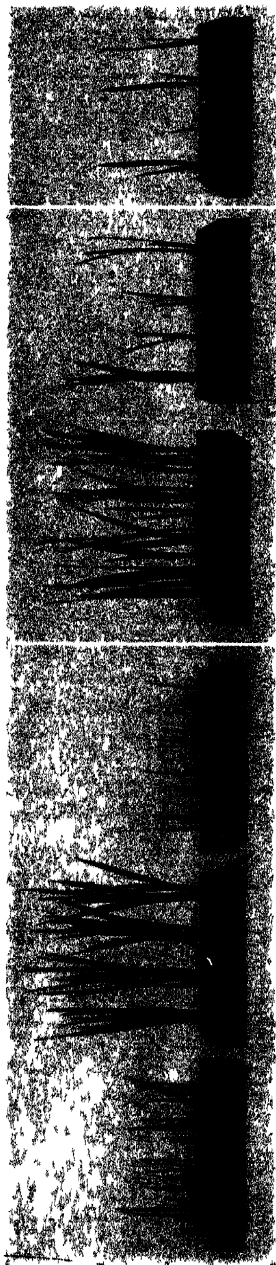
✓ The warm-temperature storage method although not successful on freshly-harvested corms gave progressively better results as the length of the rest period increased, until in the later stages the differences between it and the chlorhydrin treatment practically disappeared. In fact in one experiment storing at 30° C. for three weeks gave somewhat better results than an ethylene chlorhydrin treatment using one cc. per liter for four days. This is illustrated in text figure 6 in which the results by the two methods for Alice Tiplady, Maiden's Blush, and Souvenir are shown side by side. On the whole the growth was more satisfactory with the warm-temperature storage treatment. ✓

It must be remembered, however, that the chlorhydrin-treated lots were placed at once in soil at room temperature at the end of treatment, while the temperature-treated lots were kept at 30° C. for three weeks before being placed in soil at room temperature. If the buds could start to grow, the 30° temperature would hasten their growth; therefore, a part of the gain in growth resulted merely from the favorable effect of the higher temperature





TEXT FIG. 6. Comparison of ethylene chlorhydrin treatment and storage at 30° C. for three weeks toward end of the rest period. Left pair of flats, Alice Tiplady, center pair, Maiden's Blush, right pair, Souvenir. Left to right for each pair, ethylene chlorhydrin, one cc. per liter for four days; storage at 30° C. for three weeks.



TEXT FIG. 7. Effect of combination of warm temperature storage and treatment with ethylene chlorhydrin toward end of rest period. Variety Maiden's Blush. Left to right: ethylene chlorhydrin, two cc. per liter for two days and then planted in soil, same treatment except stored for three weeks at 30° C. after treatment and then planted in soil, ethylene chlorhydrin, one cc. per liter one day then planted in soil; same treatment except stored for three weeks at 30° C. after treatment and then planted in soil, storage for three weeks at 30° C. and then planted in soil; check, not treated.

on the growth rate, and was not directly related to the breaking of dormancy. ✓ In order to make the test more comparable an experiment was carried out in which the chlorhydrin-treated lot was divided into two portions, one being planted at once in soil and the other being packed in moist moss and stored at 30° for three weeks before being planted in the soil at room temperature. In this latter case, therefore, the corms of the temperature-treated and chlorhydrin-treated lots were held at the same temperature throughout the test, the only difference being that one lot received chemical vapors and the other did not. ✓ The result of the experiment which was carried out with corms of the variety Maiden's Blush is shown in text figure 7. ✓ It is seen that a part of the favorable effect of storage at 30° C. has been due to the more favorable conditions for rapid growth, and that corms previously treated with ethylene chlorhydrin could respond by giving more rapid forcing than was attained by the lots treated either with chemical alone or with warm-storage alone. ✓

✓ It is believed that the effect of the warm-temperature storage treatment is two-fold: first, it aids in breaking the rest period and getting the buds into condition to grow; and second, it hastens the rate of growth of buds which can grow. With freshly-harvested corms of the varieties tested in these experiments, the temperature-storage treatment was unable to bring about the first of these effects, i.e., breaking of dormancy, and consequently the second of these could not make itself felt. But at later stages in the rest period the treatment was successful in breaking dormancy and then this secondary factor, the favorable effect of the higher temperature upon the growth of buds, could become manifest. ✓

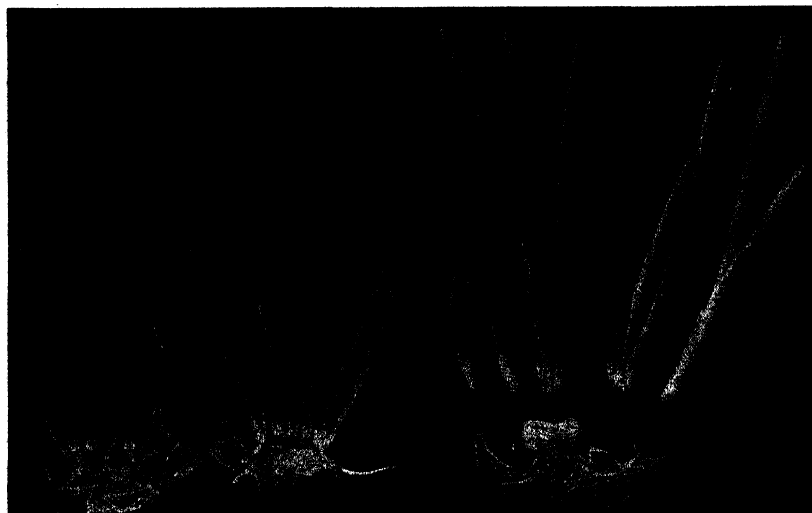
### Treatments Inducing Multiple Sprouting

✓ When sprouting begins, untreated corms usually form one, two, or three sprouts per corm. ✓ There was a tendency for the ethylene chlorhydrin treatments to induce the development of a larger number of these buds. This was especially marked in the case of Alice Tiplady even in the earlier stages of the rest period, and in the case of Remembrance in the later stages. The multiple-sprouting response is shown in text figure 8. ✓ This effect would be unfavorable for the development of good blooms, but it might be of value if it would induce extra sprouts on varieties that cannot be multiplied rapidly because of the sparseness of bulblet formation. ✓

### Development of Blooms

✓ The earliest blooms were produced by Souvenir, the first blooms opening December 20. ✓ These were from the lots harvested August 26 and treated September 2. Blooming continued until February 1. Four cc. per liter for four days, three cc. per liter for three days, and two cc. for two days all induced early blooming and gave in all 19 blooms from 48 corms. The weakest treatment, one cc. per liter for one day, was less effective, giving three blooms from 15 corms. The flowers in all cases were of good quality.

The first bloom of Alice Tiplady variety opened on January 7 in the ethylene chlorhydrin treatment, four cc. per liter for four days, but only four other bulbs out of a total of 15 produced blooms by February 1. Of the other treatments three cc. per liter for three days produced one flower but the results were negative in all other cases.



TEXT FIG. 8. Showing effect of ethylene chlorhydrin in inducing the development of a number of buds on the gladiolus corm, which usually produces one to three sprouts per corm. Variety, Alice Tiplady.

With Maiden's Blush the ethylene chlorhydrin treatment, two cc. per liter for two days, bloomed first on January 7 and other blooms late in January and early in February. In all 11 blooms were obtained from 16 bulbs. This lot received extra light by means of electric bulbs suspended above the bench.

No blooms of any variety were obtained by February 10 from any of the ether, ethylene, or warm-temperature storage lots.

It will be noted that, even with the Souvenir and Maiden's Blush treatments giving the most favorable blooming response, less than 50 percent of the plants produced blooms. An examination<sup>3</sup> of the stems of the plants that did not bloom showed that, in nearly every case, a flower-stalk had formed and had forced its way only partly up the stem; these blasted at different heights above the corm, some nearly reaching the top. This condition is called "blindness" and, according to Pridham (5), is common with gladiolus forced in the greenhouse. Pridham obtained a similar

<sup>3</sup> For this information regarding the development of flower-stalks I am indebted to Dr. Norma E. Pfeiffer who will give a further report upon this phase of the subject.

response with gladiolus corms that had passed through their natural dormant period. Consequently the "blindness" of the plants in the present experiment is not thought to indicate a deficiency in the chemical treatment of the corms, but is regarded as a normal response of gladiolus to the conditions in the greenhouse during the winter months when the days are short and the light intensity is low. The light conditions during November, December, and January, during the present test were unfavorable for growth of plants, most of the days being cloudy or foggy. It is clear that, with gladiolus, a successful method for inducing early germination of the corms could solve only part of the problem of obtaining early blooming; the work would have to be supplemented by experiments on the most favorable conditions of light, temperature, and soil for inducing the production of blooms from the plants that had been induced to grow.

#### SUMMARY

1. Freshly-harvested gladiolus corms of several different varieties were treated with vapors of ethylene chlorhydrin in order to break the rest period.

2. The results varied with the variety and with the stage of dormancy at which the treatment was applied. The varieties Souvenir, Maiden's Blush, and Alice Tiplady responded to treatments applied within about a week after harvest; corms of the Halley variety did not respond satisfactorily to treatments until about a month after harvest; with Remembrance the treatments were not successful at any stage of the rest period.

3. Treating Halley corms at once after harvest induced sprouting but the sprouts did not continue to elongate; instead, they developed secondary corms which then went back into dormancy.

4. Blooms of Souvenir were obtained in late December and early January from corms harvested on August 26 and treated on September 2. Alice Tiplady and Maiden's Blush bloomed about three weeks later than Souvenir. Halley did not produce blooms at all.

5. Not more than about 50 percent of the plants that grew furnished blooms. In such cases the non-blooming plants formed flower-stalks, but these blasted within the plant before emergence. Reasons are given for believing that this "blindness" was not induced by the chemical treatment but was caused by the unfavorable light conditions prevailing in the greenhouse during the winter months. \*

6. Ethylene chlorhydrin treatments increased the number of sprouts produced per corm, particularly of the variety Alice Tiplady.

7. Of the other treatments tried, exposure to ethylene gas and warm-temperature storage were not effective in breaking the dormancy of freshly-harvested corms, but they showed a favorable effect if applied at a later stage of the rest period.

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## THE USE OF CARBON DIOXID FOR PROLONGING THE LIFE OF CUT FLOWERS, WITH SPECIAL REFERENCE TO ROSES<sup>1</sup>

NORWOOD C. THORNTON

For many years investigators have reported various treatments which were effective in increasing the life or preserving the color and appearance of cut flowers. These treatments have usually consisted in the addition of one or more chemicals to the water in which the flower stems were placed. Farrington (2), Fourton and Ducomet (3), Grinstead (4), and Laurie (7) reported chemical treatments that were favorable in prolonging the life of cut flowers. Knudson (6), however, in studying the effect of chemicals on flowers of relatively short duration of life, was unable to substantiate the results of the French workers. Hitchcock and Zimmerman (5) found no marked beneficial results from the use of fifty different chemical treatments many of which have been favorably reported on by other investigators. These authors as well as Perret (8) have shown, however, that low temperature and relatively high humidity are effective in prolonging the life of cut flowers.

So far as the writer was able to determine from the available literature no attempt has been made to prolong the life of cut flowers by altering the composition of the atmosphere about them. Investigating carbon dioxide storage of fruits, West (9) found that the life of apples was increased by one-third to one-half when held in 12-15 percent carbon dioxide. Brooks, Cooley, and Fisher (1) found that 100 percent carbon dioxide for six days produced a very decided inhibition of the activities of the apple so that in storage it developed color more slowly than the untreated apple.

In some recent experiments the writer found that in carbon dioxide storage, fruits, vegetables, and flowers were affected in various ways, depending upon the concentration of gas, the temperature, and the variety of fruit, vegetable, or flower. Preliminary tests with flowers indicated that, in the case of the rose, carbon dioxide retarded bud opening and prolonged the life of the flower. The purpose of this paper is to report the results of experiments dealing with the effect of carbon dioxide on cut flowers, principally roses. Other gases such as hydrogen, nitrogen, etc. have not yet been studied. A preliminary test with oxygen indicated that this gas hastened the maturing processes in rose flowers.

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

## MATERIAL AND METHODS

Rose buds of the varieties Mrs. F. R. Pierson, Briarcliff, Talisman, Double White Killarney, and Mme. Butterfly were procured from local florists. The roses were usually received the day that they were cut, though at no time were the flowers more than one day old at the beginning of the treatment. Rose buds were selected for freedom from injury and discolorations and only similar flowers were used.

Roses were held in carbon dioxide for periods varying from twelve hours to seven days in order to observe the effect of length of storage, temperature, moisture, and the concentration of carbon dioxide on the life of the flower. In a few tests the roses were held at temperatures ranging from 32° to 60° F., but the majority of the tests were made at either 38° or 50° F., since it is within this temperature range that most of the commercial florists keep their flowers. Before placing in storage, the flower stems were wrapped in a moist paper towel around which wax paper was folded so as to prevent drying. After removal from the storage cans, the roses were held in an inside unheated room at 75° F. (with stems in water) in order to observe the opening of the buds and the time of petal fall.

The concentrations of carbon dioxide used in all tests were between 5 and 80 percent in steps of five up to 30 percent and in steps of ten from 30 to 80 percent. The roses were sealed in 35-liter tin cans (size  $12\frac{1}{2} \times 18$  inches) during treatment. The seams of the cans were closed with paraffin and the tops were sealed with plasteline clay. The desired concentration of carbon dioxide was obtained by placing a weighed amount of commercially manufactured "Dry Ice" (solid carbon dioxide) in a dish suspended inside of the can one inch from the top. The weight of "Dry Ice" used (3.5 gm. for 5%, 6.5 gm. for 10%, and 11 gm. for 15%) was not sufficient to alter the temperature in the can for an appreciable length of time. Gas analyses for the determination of the carbon dioxide content of the atmosphere about the roses were made at the beginning and at the end of the test period. For periods longer than one day the first analysis was taken twenty-four hours after the roses were placed in storage and the last one made as the roses were removed. For shorter storage periods the analyses were made immediately before the flowers were removed from storage.

In view of the possibility of a commercial application of the carbon dioxide treatment to roses or to other flowers, an attempt was made to duplicate some of the conditions to be dealt with. It was estimated that the average florist's case would be closed tightly for a period not exceeding sixteen hours each day and opened many times during the next eight hours. During the sixteen hour period enough carbon dioxide could be held in the case to aid in keeping the flowers, but during the eight hour period it would be very difficult to maintain a desirable concentration. With these considerations in mind a test was made exposing the rose buds to various concentrations of carbon dioxide in sealed cans during the sixteen hour period, then removing

them from the can for the next eight hours. This alternated carbon dioxide-air treatment was carried on for fourteen days at a constant temperature of 42° F., which is a temperature desired by commercial florists. In this test the usual commercial handling was given the roses; the stems were cut each day and kept in water during the entire period, but no petals were removed.

It was necessary to refer to the bud opening in terms of the degree of unfolding of the petals such as one-quarter, one-half, and completely open. A completely open flower that was either wilted or had dropped petals was considered a worthless flower. The higher concentrations of carbon dioxide caused injury to the petals by either browning the edges or discoloring the veins at the base. The discolored veins stood out very markedly against the normal flower color. Injury other than the killing effect was evident by a change in color whereby a pink rose (Briarcliff) became grey or nearly white and a red rose (Mrs. F. R. Pierson) turned either somewhat blue or pink. All observations on the opening of the treated buds were recorded with reference to the dropping of petals or to the wilting of the check flowers.

### RESULTS

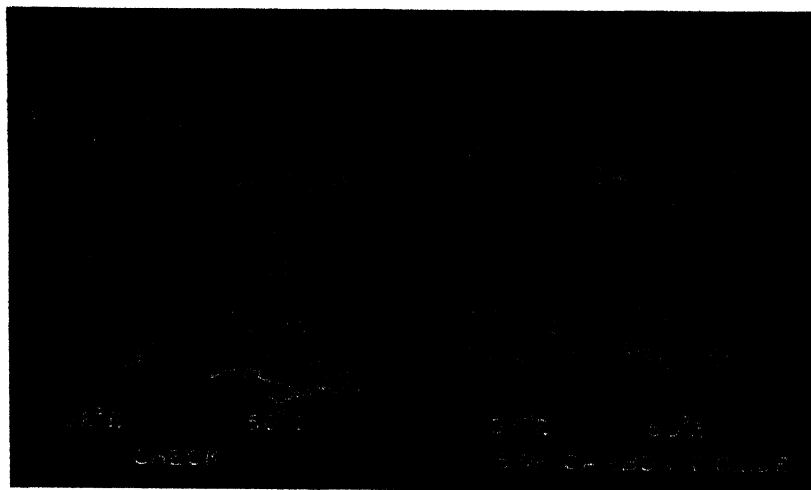
When the period of storage was longer than one day, roses were benefited by carbon dioxide treatment within the range of 5-15 percent. Higher concentrations caused injury to Briarcliff and Mrs. F. R. Pierson, varying from slight at 20-25 percent to considerable at higher percentages. The Talisman rose buds were benefited by concentrations of carbon dioxide up to and including 30 percent, but were injured by 40 percent. Treated buds in all beneficial concentrations of carbon dioxide lasted from twelve hours to two days longer than the check flowers. Beneficial effects were most noticeable when the roses were held at 50° F. for seven days of storage. The check rose buds held at 50° F. for seven days usually opened one-half to three-quarters, then lost petals, while the treated ones remained nearly the same as they were at the beginning of the experiment. The lower the temperature, the less effective was the treatment during storage since at low temperatures the untreated buds were retarded in opening. However, upon removal to warm air the untreated buds opened and lost petals much more quickly than the treated ones.

### Continuous Carbon Dioxide Treatment

Text figure 1 shows the effect of temperature and carbon dioxide on Briarcliff rose buds. Untreated buds held for seven days at 38° F. opened very little as compared with those held at 50° F., and in addition the latter had lost many petals. The untreated buds sealed in cans during the storage period at 38° or 50° F. produced only 1.5 percent carbon dioxide which was not sufficient to alter their opening. Five percent carbon dioxide was beneficial for the buds held at 50° F. during the storage period. The treated



buds had opened less than the checks and failed to lose petals even upon vigorous shaking, but at the end of one day in warm air (75° F.) petal fall was noticeable. The visible effectiveness of the carbon dioxide treatment as seen in text figure 1 was not evident during storage at 32° F., but when removed to warm air (75° F.) the treated buds opened slowly and remained in good condition from one to two days longer than the check buds.



TEXT FIG. 1. The effect of temperature and 5 percent carbon dioxide on Briarcliff rose buds held for seven days at 38° and 50° F.

*Seventeen-day Storage Period.*—Storage of Briarcliff rose buds in carbon dioxide at 38° F. for seventeen days failed to give the beneficial results obtained with shorter periods of treatment. The check rose buds lost petals before the end of the storage period, while the buds treated with 3 to 8 percent carbon dioxide opened approximately one-half. Although higher concentrations (13 percent) of carbon dioxide retarded the opening of the buds, bleaching and other types of injury resulted. Even though the treatment was effective in prolonging the life of the roses while in storage, this beneficial effect was lost because of the fact that the buds opened rapidly and lost petals within a few hours after removal to warm air (75° F.). These results show that a shorter period of storage is necessary if the check roses are to remain in a fair condition upon removal to warm air at 75° F.

*Seven-day Storage Period.*—Tests in which Briarcliff, Mrs. F. R. Pierson, and Talisman rose buds were held for seven day periods gave similar results for the three varieties. Briarcliff roses held in all concentrations of carbon dioxide were retarded in opening as shown in part by table 1 and Plate XXXVI, figures 1 and 2. Buds treated with 5–15 percent carbon dioxide inclusive were not injured, but those held in 20 percent carbon dioxide re-

TABLE 1. *Effect of Carbon Dioxid on Briarcliff Rose Buds Stored Seven Days at Different Temperatures*

Treatment	32° F.	38° F.	50° F.	60° F.
Check	B	B	E	X
5% CO <sub>2</sub>	A	A	B	X
10% CO <sub>2</sub>	A	A	A	X
15% CO <sub>2</sub>	A	A	A	X
20% CO <sub>2</sub>	D	D	D	X
25% CO <sub>2</sub>	P	P	D	X
30% CO <sub>2</sub>	P	P	P	X

A, good buds not more than 1/4 open.

B, good buds 1/2–3/4 open.

D, buds that have outer petals slightly injured or off color—easily removed.

E, buds open, few petals falling.

P, buds greatly injured.

X, buds wilted—impossible to revive.

ceived injury to the outermost petals. Increasing the concentration to 25 percent caused browning of the petal edges and bleaching. Other types of injuries to the buds resulting from high concentrations of carbon dioxid were discoloration of the veins of the petals, extreme bleaching, and softening of the stems. Concentrations of carbon dioxid bringing about beneficial or injurious effects to the rose buds at 38° F. gave similar results at 50° F. A given concentration that was slightly injurious at 50° F. was greatly injurious at 32° F. At the other extreme of the temperature range (60° F.) there was practically no beneficial effect of the treatment.

Like the Briarcliff, the Mrs. F. R. Pierson and Talisman rose buds held at 50° F. and the Mrs. F. R. Pierson at 38° F. were worthless at the end of the storage period, as shown in table 2. In contrast to these results the buds treated with 5–15 percent carbon dioxid were in good condition when removed from storage and remained so for one to two days when held in warm air. The Mrs. F. R. Pierson rose buds were slightly injured by 20 percent carbon dioxid, the outer petals having blue spots. As the concentration was increased the injury became more conspicuous. Unlike the other varieties, Talisman rose buds were benefited by 30 percent carbon dioxid without injury as shown in table 2 and Plate XXXVI, figure 3. Increasing the concentration to 40 percent injured the edges of the petals and the buds would not open completely when held under favorable conditions at 75° F. The Talisman rose buds vary somewhat as is evident in figure 3 where a few buds did not respond to the treatment. In general the buds were retarded from opening by concentrations of carbon dioxid greater than 5 percent.

TABLE 2. *Effect of Carbon Dioxid on Rose Buds Stored at Different Temperatures*

Treat- ment	Days in Storage	32° F.	38° F.		42° F.	50° F.			60° F.
		Briar- cliff	Pier- son	Briar- cliff	Talis- man	Pier- son	Briar- cliff	Talis- man	Briar- cliff
Check	3	B		B	B			C	
	7	B	E	B	C	O	E	O	X
5% CO <sub>2</sub>	7	A	C	A	B	E	B	B	X
10% CO <sub>2</sub>	3	A		B	A			B	
	7	A	B	A	B	E	A	B	X
15% CO <sub>2</sub>	3	A		A	A			A	
	7	A	A	A	A	C	A	A	X
20% CO <sub>2</sub>	3	D		D	A			A	
	7	D	D	D	A	D	D	A	X
25% CO <sub>2</sub>	3	D		D	A			A	
	7	P	P	P	A	P	D	A	X
30% CO <sub>2</sub>	3	D		D	A			A	
	7	P	P	P	A	P	P	A	X
40% CO <sub>2</sub>	3	P		P	A			A	
	7				P			P	
50% CO <sub>2</sub>	3	P		P	D			D	

A, good buds not more than 1/4 open.

B, good buds 1/2-3/4 open.

C, buds open, no petals falling.

D, buds that have outer petals slightly injured or off color—easily removed.

E, buds open, few petals falling.

O, worthless flowers, fully open petals falling or wilting.

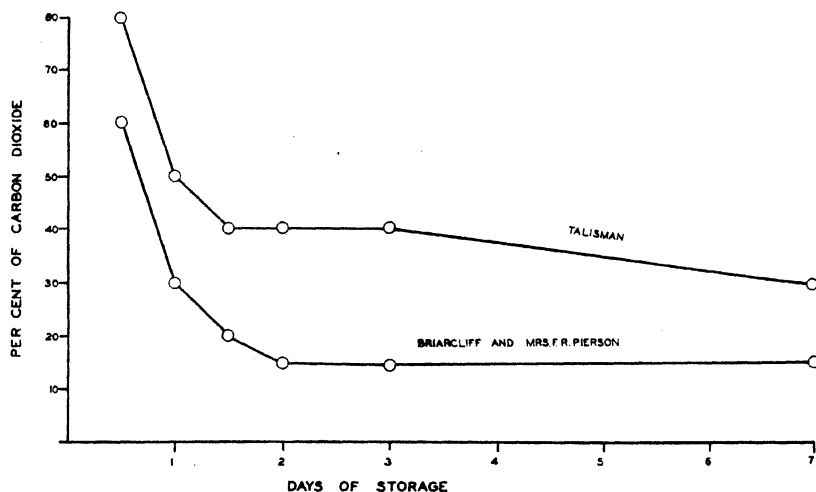
P, buds greatly injured.

X, buds wilted—impossible to revive.

*Three-day Storage Period.*—Shortening the storage period to three days altered only slightly the beneficial effect of carbon dioxid on the rose buds. The shorter period, however, changed the range of the injurious concentration of the gas to some extent as shown in table 2. Twenty percent carbon dioxid slightly injured the Briarcliff rose buds while 30 percent brought about a definite injury to only the outermost petals. Higher concentrations caused petal, bud, and stem injury as previously described.

Plate XXXVII, figure 4 shows the effect of various concentrations of carbon dioxid on Briarcliff rose buds held three days at 38° F. These

roses were photographed three hours after they had been removed from storage to warm air (75° F.). Figure 5 shows the same roses as in figure 4 after they had been held in the warm air (75° F.) for one day. The check roses at this time were losing petals, while the treated ones were in good condition at various stages of opening. The buds exposed to 15 percent or higher concentrations of carbon dioxide remained in good condition for two days after the check had begun to drop petals.



TEXT FIG. 2. Maximum concentrations of carbon dioxide that prolonged the life of the rose buds without causing injury during various periods of storage at 38° and 50° F.

Talisman roses held in storage at 42°–50° F. for three days were benefited by concentrations of carbon dioxide up to 40 percent, but were injured with higher concentrations. Buds held in 10–15 percent opened fairly rapidly and lasted one day longer than the checks, while those treated with concentrations of 20–40 percent lasted from two to three days longer than the checks.

*Twelve-hour to Two-day Storage Periods.*—As shown in text figure 2 the range of favorable concentrations of carbon dioxide on the three varieties of rose buds held at 38°–50° F. remained the same for storage periods of two to three days. For the Briarcliff and Mrs. F. R. Pierson 15 percent gave favorable results without causing injury. Higher percentages of carbon dioxide during this period of storage caused injury to the rose buds. The Talisman rose buds were benefited by 40 percent and injured by higher concentrations of carbon dioxide. Shortening the period of storage to thirty-six hours or less allowed a very marked increase in the concentration of carbon dioxide that would not cause injury to the rose buds. The Briarcliff and Mrs. F. R. Pierson roses were benefited by 60 and injured slightly by

80 percent carbon dioxide during the first twelve hours of storage. The Talisman variety showed no injurious effects at any concentration during this period. For the various storage periods tested at temperatures of 38° and 50° F., text figure 2 shows that roses withstand a high concentration of carbon dioxide for only a short period, but a relatively low concentration for a long period gives more beneficial results.

The opening of the rose buds stored for twelve hours was retarded by a range of 20-80 percent carbon dioxide. These concentrations aided in the keeping of the roses approximately twelve hours longer than the check. The buds in lower concentrations than 20 percent opened and dropped petals in a similar manner to the checks. Increasing the storage period from twelve to twenty-four hours decreased the maximum concentration that was beneficial without injury. Favorable concentrations (20-30 percent) for this period prolonged the life of the roses one day. For a thirty-six hour storage period the roses receiving a 20 percent carbon dioxide treatment lasted one and one-half days longer than the checks. Even the 10 percent carbon dioxide treatment was effective in the thirty-six hour storage in which case the roses lasted approximately twelve hours longer than the checks. In the forty-eight hour storage period the treated roses lasted as much as two days longer than the checks. The lasting quality of the Talisman rose was usually difficult to judge due to its habit of opening without losing petals. Because of this characteristic the lasting qualities of the treated buds were based upon wilting tendency.

### Alternated Carbon Dioxide-Air Treatment

Five varieties of rose buds were used for the alternated carbon dioxide-air treatment. This treatment consisted in keeping the flowers in various concentrations of carbon dioxide for sixteen hours and in air for eight hours each day. Observations were made after three, eight, and fourteen periods of treatment. At the end of the third and fourteenth period of treatment a set of flowers was removed to warm air (75° F.) to observe bud opening. Check buds in air were held in sealed cans for the same periods as the treated buds.

*Three Periods of Treatment.*—The effect of alternated carbon dioxide-air treatment on the roses observed at the end of the third period compared favorably with the results of the two day continuous exposure test. The Mrs. F. R. Pierson rose buds held in 20 percent carbon dioxide developed a few blue spots on the outer petals. A concentration of 30 percent or more caused browning of the edges of the outer petals of the Double White Killarney and produced bleaching and bluing of the Briarcliff and Mrs. F. R. Pierson. The Talisman and Mme. Butterfly roses did not appear to be injured by any concentration of carbon dioxide used.

After removal to warm air (75° F.) the check buds opened rapidly and lasted only one day. Buds treated with 5 percent carbon dioxide remained

in good condition for only a few hours longer than the checks. The roses which were held in concentrations of 10 percent or higher remained in good condition for two days. The Talisman and Mme. Butterfly rose buds held in 40 percent or higher concentrations of carbon dioxid opened slowly into nicely shaped and normal colored flowers by the end of the second day in warm air. At the beginning of the third day all rose buds had opened and in most cases petals were falling.

*Eight Periods of Treatment.*—As had been observed in former tests the carbon dioxid injury to the rose buds became more definite as the period of treatment was lengthened. After eight periods of treatment all the roses held in 30 percent and higher concentrations were injured. Roses in the lower concentrations of carbon dioxid were greatly benefited by the treatment. At this time photographs were taken of all roses except the Mrs. F. R. Pierson and Double White Killarney which did not show such marked favorable effects. Plate XXXVIII, figures 6, 7, and 8 show the effect of the carbon dioxid treatment on the three varieties of roses, Briarcliff, Mme. Butterfly, and Talisman. In every treatment carbon dioxid retarded the opening of the buds. The Briarcliff rose buds shown in figure 6 were greatly benefited without injury by 5-17 percent, inclusive, and were only slightly injured on the outermost petals by 20-23 percent carbon dioxid. The Mme. Butterfly rose, shown in Plate XXXVIII, figure 7, was benefited by 20-23 percent carbon dioxid without causing injury though the outer petals had a tendency to curl. Talisman roses shown in figure 8 were retarded from opening by 5-11 percent concentrations of carbon dioxid, whereas the check buds had opened and had lost some petals (fallen petals not shown in the photograph).

*Fourteen Periods of Treatment.*—Fourteen periods of the alternated treatment using 20-23 percent carbon dioxid resulted in definite injury to the Briarcliff, Mrs. F. R. Pierson, and to the outer petals of the Double White Killarney. Lower concentrations caused no noticeable injury to the rose buds. The rose buds removed from the cans at the end of fourteen days were divided into two lots, one lot remaining at 42° F. in air and the other lot being placed in air at 75° F. At this time the check buds had opened considerably, but in the course of two to three hours those held at 75° F. lost many petals, whereas those held at 42° F. lasted two days. Roses treated with 9-23 percent carbon dioxid opened from one-quarter to three-quarters during treatment. When held in air at 42° F. they lasted from three to four days, whereas those removed to warm air (75° F.) lasted only twelve hours. The rapid maturing of rose buds appears to be characteristic of a sudden change to a high temperature after a long period of storage at a low temperature.

### Carbon Dioxid Treatment of Other Flowers

Flowers other than roses were held in storage for periods of four and five days with various concentrations of carbon dioxid. The gas treatment

prolonged the life of the flowers that were in the bud stage, but appeared to have no beneficial effects on the open ones. Gladiolus (two varieties, color, salmon-pink and rose-pink) and buds of the snapdragon, *Antirrhinum majus* Linn. (various colors), were retarded from opening by 15 percent carbon dioxide when held in storage four days at 38° and 50° F. When removed to warm air (75° F.) the treated flower buds opened much more slowly without loss of color and lasted longer than the checks. Increasing the concentration of carbon dioxide (20 percent on the rose-pink and 25 percent on the salmon-pink gladiolus and the snapdragon) caused bleaching of the buds already open and injury to those closed. When removed to warm air (75° F.) the injured buds opened about one-half with poor shape and in most cases with a wilted appearance. The bleaching effect did not extend to the closed buds except in very high concentrations of the gas which also darkened the buds and injured the spike. The cosmos held in 15 percent carbon dioxide four days at 50° F. did not lose petals as easily as the checks. At a higher concentration of 25 percent, both the stem and petals of the cosmos were injured. The Jersey's Beauty dahlias held four days at 50° F. were not noticeably altered by 10 percent, but they were bleached and injured by 15 percent and higher concentrations of carbon dioxide.

Carnations and sweet peas (*Lathyrus odoratus* Linn.) were included in the alternated carbon dioxide-air treatments. The carnation, variety Matchless, was greatly benefited by 5-11 percent and slightly benefited by 15-17 percent carbon dioxide as compared with the check. Other varieties, Surprise and Betty Lou, showed some beneficial effects of the carbon dioxide treatment. Carnations treated with low concentrations of carbon dioxide usually did not close or become ragged as quickly as the checks, while higher concentrations of carbon dioxide promoted the development of a ragged condition of the flowers during storage. Sweet peas, on the other hand, did not show any beneficial effects of the carbon dioxide treatment.

### Carbon Dioxide Treatment of "Ferns"

Further variation in response to the carbon dioxide treatment was observed in the tests made on two varieties of ferns used by florists. The broad frond fern *Aspidium spinulosum* var. *intermedium* (O. F. Muller) Sw. was held seven days at 50° F. in 15 percent carbon dioxide without any apparent change, but was killed by a concentration of 25 percent. "Asparagus fern," *Asparagus plumosus* Baker, was apparently not affected by any concentration of carbon dioxide used. None of the fern tests showed any beneficial effects of the treatment over that of the check ferns.

### DISCUSSION

From a commercial standpoint the beneficial effects of a carbon dioxide treatment for prolonging the life of rose buds may become of considerable value. Florists usually do not sell roses for home use that have been in the

refrigeration case more than three days. On the other hand rose buds subjected to carbon dioxid treatment remain in good condition for seven days. After a seven-day treatment these roses lasted fully as long at room temperature as the untreated ones that had been removed from normal storage at the end of three days. From these results it is seen that roses may be kept in carbon dioxid storage an additional four days. These beneficial results apply to roses held in 15 percent carbon dioxid at either 38° or 50° F.

Storage temperatures of 38° and 50° F. were selected as being the extremities of the range used by commercial florists. Roses held for any period of storage at 38° F. usually opened a little more slowly than those held at 50° F., but the effects of the carbon dioxid treatment were found to be approximately the same at either temperature. This did not hold true for treatments at 32° F. in which case injuries for a given concentration were much more pronounced. For example, 25 percent carbon dioxid at 38° or 50° F. caused slight bleaching and browning of petals whereas at 32° F. the buds were greatly bleached, veins were discolored, and considerable browning was evident.

Rose buds only slightly injured by carbon dioxid (20 percent for seven days and 20-25 percent for three days) could no doubt be used by the florist. After the outer three or four petals that were either browned, bleached, or rolled had been removed, according to the present commercial practices, the buds opened slowly and lasted slightly longer than those treated with 15 percent carbon dioxid. Rose buds greatly injured by carbon dioxid in any period of storage were of no commercial value. Talisman rose buds when slightly injured by carbon dioxid did not open as well or last as long as the check buds. The individual variation in Talisman rose buds was much greater than in any of the other varieties used regardless of whether or not they had received carbon dioxid treatment. This varied response of different varieties of roses to carbon dioxid treatment is of sufficient interest to warrant further investigation if a commercial application is made of this work.

The effectiveness of the carbon dioxid treatment in keeping the roses in the bud stage during storage was somewhat altered by moisture conditions. Rose buds with stems held in a moist condition (stems wrapped in moist paper towel that was covered with wax paper) did not open as much as those held with stems in water. In repeated tests where all conditions, except moisture, were the same, it appeared that holding rose stems in a moist condition retarded the opening of the buds. However, it was not necessary to hold these buds in a high humidity to prevent wilting when removed to warm air after seven days of storage. Rose buds held with stems in water during storage at 38° or 50° F. opened slightly, even when exposed to fifteen percent carbon dioxid. Although the carbon dioxid is very effective in holding the roses in the bud stage regardless of moisture con-



ditions, it is an additional advantage to have the stems wrapped in moist paper. It appears that the florist might hold the excess stock of rose buds with stems in a moist condition in carbon dioxid storage at 38° to 50° F. for three to seven days. At the end of the storage period the buds could be removed to the show case or could be sold direct to the trade. Although for these experiments rose buds were stored in small containers, it is apparent that in large storage boxes the air must be constantly stirred in order to maintain a uniform concentration of the gas.

### SUMMARY

1. Carbon dioxid treatment was effective in prolonging the life of cut roses under the following conditions:

- a. When stored for three to seven days at 38° and 50° F. in a concentration of carbon dioxid between 5 and 15 percent for Briarcliff and Mrs. F. R. Pierson, and between 5 and 30 percent for Talisman.
- b. When subjected to an alternated carbon dioxid-air treatment (16 hours in 5-17 percent carbon dioxid and then eight hours in air at 42° F.) for three, eight, or fourteen days.

2. Carbon dioxid storage of rose buds should not be greatly in excess of seven days which is approximately the limit for untreated roses to be held at 38° to 50° F. Roses in carbon dioxid storage for longer periods remained in the bud stage, but upon removal to warm air they lost petals very rapidly.

3. Carbon dioxid retarded the opening of rose buds, this effect being more pronounced as the concentration of carbon dioxid was increased. Treated roses lasted four days longer in storage and from twelve hours to two days longer after removal from storage than the untreated roses.

4. Rose buds opened more slowly when their stems were wrapped with moist paper than when the stems were kept in water.

5. Carbon dioxid treatment appeared to be more effective for flowers in the bud stage than for those which were already open.

6. Injuries due to high concentrations of carbon dioxid for storage periods exceeding two days were as follows:

- a. Outer three or four petals slightly bleached and discolored or curled.
- b. Many petals discolored such as bleaching, browning, or bluing.
- c. Failure of buds to open, discoloration of petal veins, and softening of stems.

7. Roses subjected to excessive concentrations of carbon dioxid for too long a time suffer the injuries described under paragraph 6. Roses stored under the conditions recommended in paragraph 1 above showed no injuries. It is possible that the limits of concentration and time may be somewhat extended when injured outer petals are removed from the buds.

The writer is greatly indebted to the Dry Ice Corporation of America for furnishing funds and for making helpful suggestions toward the carrying on of this investigation.

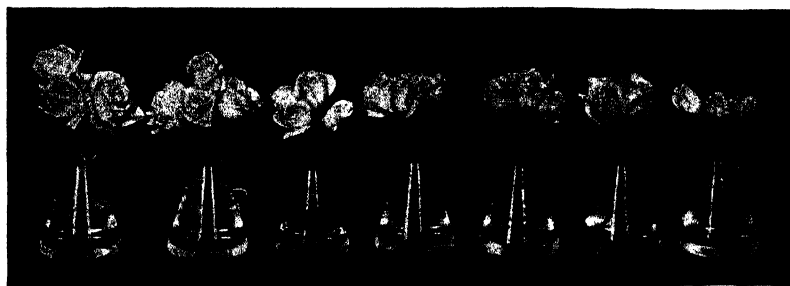
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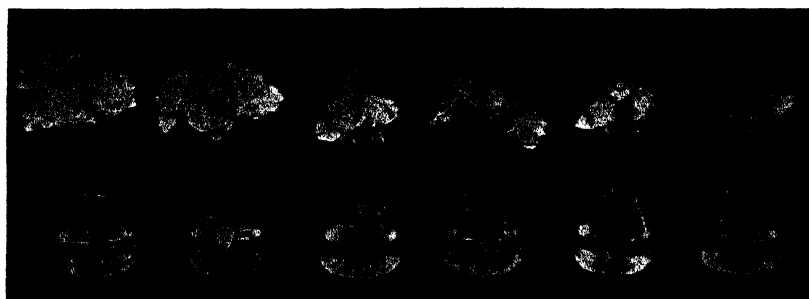




CHECK 5% 10% 15% 20% 25% 30%  
FIG.1.EFFECT OF CARBON DIOXIDE ON BRIARCLIFF ROSES  
HELD 7 DAYS AT 38°F.

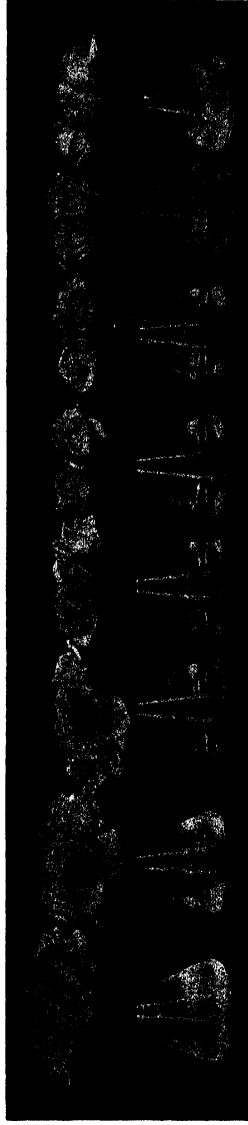


CHECK 5% 10% 15% 20% 25% 30%  
FIG.2.EFFECT OF CARBON DIOXIDE ON BRIARCLIFF ROSES  
HELD 7 DAYS AT 50°F.

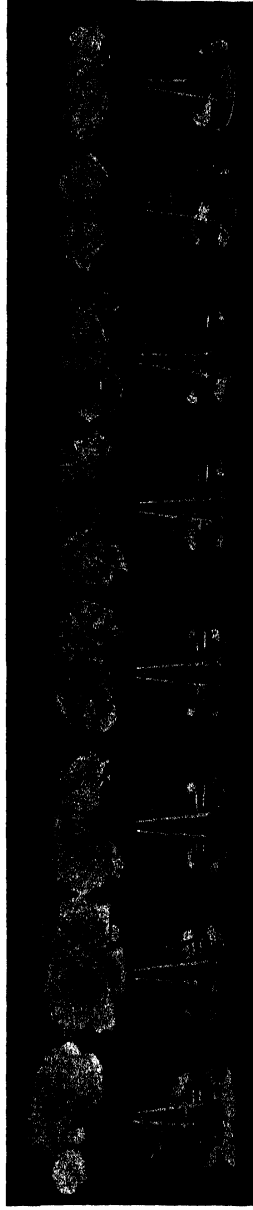


CHECK 5% 10% 15% 20% 30%  
FIG.3.EFFECT OF CARBON DIOXIDE ON TALISMAN ROSES  
HELD 7 DAYS AT 42°F.





CHECK 10% 15% 20% 25% 30% 40% 50%  
 FIG. 4. EFFECT OF CARBON DIOXIDE ON BRIARCLIFF ROSES  
 3 DAYS AT 38°F. THEN IN AIR 3 HRS. BEFORE PHOTOGRAPHING.  
 (SEE FIG. 5)

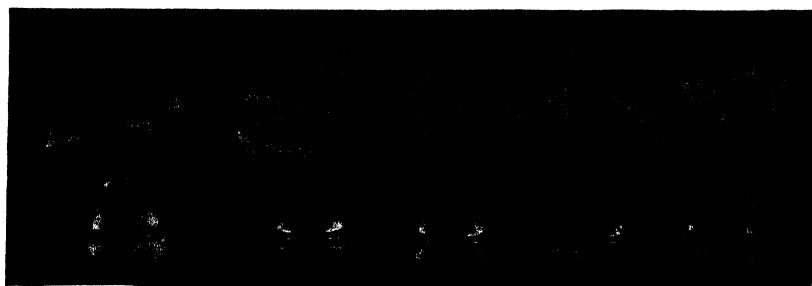


CHECK 10% 15% 20% 25% 30% 40% 50%  
 FIG. 5. ROSES SHOWN IN FIG. 4 AFTER BEING HELD 1 DAY AT 75°F

THORNTON: LIFE OF CUT FLOWERS



THREE VARIETIES OF ROSE BUDS HELD AT 42°F. UNDER ALTERNATED CARBON DIOXIDE-AIR TREATMENT. (CARBON DIOXIDE FOR 8 PERIODS OF 16 HOURS EACH AND AIR FOR 9 PERIODS OF 8 HOURS EACH)



CHECK      5%      9-11%      15-17%      20-23%  
FIG. 6 BRIARCLIFF



CHECK      5%      9-11%      15-17%      20-23%  
FIG. 7 MME. BUTTERFLY



CHECK      5%      9-11%  
FIG. 8 TALISMAN





## LOCAL LESIONS ON BEAN LEAVES INOCULATED WITH TOBACCO MOSAIC VIRUS<sup>1</sup>

W. C. PRICE

### INTRODUCTION

Necrotic lesions appear at the points of inoculation when tobacco mosaic virus is rubbed over the surfaces of leaves of a number of species of *Nicotiana*. Holmes (1) studied the development of such lesions in five different *Nicotiana* species. Lesions occurring on *N. glutinosa* make their appearance the second day after inoculation and are well developed on the fourth or fifth day. Since their number is largely determined by the concentration of virus in the inoculum, the lesions have been made the basis of a method for the rapid determination of virus concentration.

The purpose of this paper is to describe briefly similar necrotic lesions which occur on the leaves of certain varieties of the common garden bean, *Phaseolus vulgaris*, when they are inoculated with the virus of tobacco mosaic, and to suggest a possible use of these lesions in measuring virus concentration.

In securing virus for use as inoculum, care was taken to obtain samples that contained no virus other than that of tobacco mosaic. Samples of virus obtained from several different sources were used. All these samples resulted in the production of similar lesions on inoculated leaves of susceptible bean plants. The number of lesions produced on the leaves was roughly proportional to the concentration of virus used as inoculum. There was a multiplication of the virus in leaves on which local lesions developed. It is therefore believed that the disease described was caused by the virus of tobacco mosaic.

### METHOD

The method of inoculation used in the experiments reported herein was similar to that used by Holmes in his work on *N. glutinosa*. A cheese-cloth pad, saturated with virus solution, was rubbed once over the entire upper surface of each bean leaf. The inoculated leaves were washed immediately with tap water in order to remove any excess virus.

Plants were grown in pots for eight to twelve days before inoculation. In general, the first young compound leaves were just appearing at this time.

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

Pea (navy) bean seed used in these experiments was obtained from a local grocery store. For this reason, the pea bean was treated separately and is not considered here as a distinct variety. Seed of the 77 varieties listed in table 1 was secured from four reliable seedsmen.

#### DEVELOPMENT OF LOCAL LESIONS ON THE PEA (NAVY) BEAN

The pea bean was used in the first attempt to inoculate leaves of bean plants with tobacco mosaic virus. Simple leaves of 14 pot-grown plants of the pea bean were inoculated soon after the first compound leaves made their appearance. The source of inoculum was a pure strain of the common field type of tobacco mosaic diluted 1 : 20 with water and kept frozen at  $-6^{\circ}$  to  $-10^{\circ}$  C. for four years. Two days after inoculation, 50 to 200 necrotic lesions appeared on each of the inoculated leaves. These lesions were one-half millimeter in diameter or smaller and each consisted of a pale necrotic area surrounded by a ring of dark red tissue. They increased slightly in size but never became much larger than one-half to one millimeter in diameter. Six similar plants inoculated in the same manner but with water as inoculum did not develop lesions. Sixteen other bean plants inoculated with juice from healthy tobacco plants remained free of lesions. Similarly, four uninoculated plants remained free of lesions. To confirm this result, 12 plants were inoculated with an undiluted virus extracted from diseased *N. tabacum* plants. All developed lesions two days later. Eight plants inoculated with water and four plants uninoculated did not develop lesions. These experiments indicate that tobacco mosaic can be transmitted to the pea (navy) bean. Experiments on the transmission of the virus of tobacco mosaic from beans to tobacco are discussed in another part of this paper.

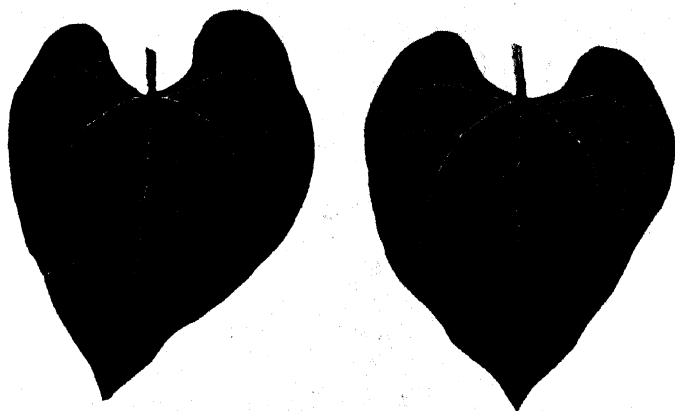
#### VARIETAL SUSCEPTIBILITY

Transmission of tobacco mosaic virus to pea beans immediately suggested the possibility of transmission to other varieties of beans. Consequently, 77 commercial varieties of *Phaseolus vulgaris* were tested for susceptibility and resistance. Plants for this test were grown in four-inch pots until the first compound leaves had fully developed. The simple leaves, which are the first to develop above the cotyledons, were inoculated with an undiluted virus secured from mosaic *N. tabacum* plants that had become infected three weeks earlier from inoculation with a 1 : 100,000 dilution of tobacco mosaic virus. The results of this test are presented in table 1, in which the number of plants of each variety inoculated, the number of plants on which lesions appeared, and the number of check plants are listed. These varieties are separated into three groups depending upon their susceptibility. In the first group, every plant inoculated developed local necrotic lesions; in the second group, lesions appeared on one or more plants of each variety inoculated; in the third group, none of the plants inoculated developed lesions and all were apparently healthy at the end of

six days. Plants in the third group were reinoculated on the sixth day in order to detect any extremely resistant variety not infected in the first attempt. Compound leaves were now inoculated with virus from the same source as that used in the first attempt. With one exception, all the plants reinoculated remained healthy for as long as 14 days. This exception was one of 16 reinoculated plants of the variety Kentucky Wonder which developed numerous necrotic lesions on the inoculated leaf. An explanation for this behavior has not been found but it is possible that seed of the variety was impure or not true to type.

Part of the above experiment was repeated using tobacco mosaic virus from a different source and the 15 varieties found to be susceptible. Plants of each variety were inoculated with an undiluted virus which had been frozen at  $-6^{\circ}$  to  $-10^{\circ}$  C. for more than two weeks. The results of this experiment, presented in table 2, confirm those of the first test.

In addition, plants of *Vicia faba* and *Vigna sinensis* were tested at the



TEXT FIG. 1. Typical lesions produced on leaves of Scotia bean following inoculation with tobacco mosaic virus. The leaf at the right was not inoculated.

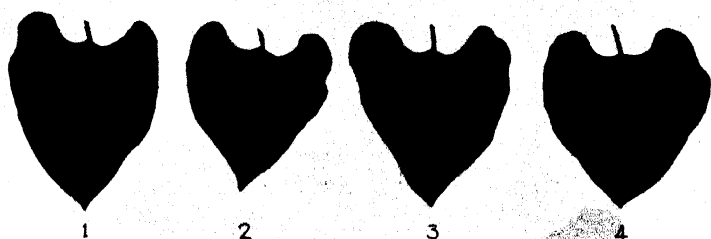
same time as plants listed in table 1. No lesions were developed on plants of either of these species.

Lesions on all susceptible varieties resembled in appearance those described on pea beans. The lesions varied slightly in size and considerably in number on the different varieties. In some varieties, only eight or ten lesions were produced on each inoculated leaf; in others, several hundred lesions developed on each leaf. Text figure 1 shows typical lesions produced on the variety Scotia.

Infected plants were under observation for at least three weeks during which time no indication of a systemic infection was noticed. Leaves formed after plants had been inoculated developed neither mottling nor necrotic lesions and appeared normal in every respect.

#### TRANSFER OF VIRUS FROM BEAN TO TOBACCO

An attempt was made to transfer tobacco mosaic virus from the lesions on bean leaves to tobacco plants. The inoculum used for this test was obtained from varieties which exhibited a large number of lesions on inoculated leaves. An infected leaf was folded in cheesecloth, pounded to express the juice, and rubbed over the upper surface of each leaf of one or more *N. tabacum* plants and one *N. glutinosa* plant. Check plants were treated in exactly the same way except that the inoculum used was obtained from inoculated leaves of varieties which did not develop necrotic lesions. The results of this experiment are shown in table 3. Since all the leaves used



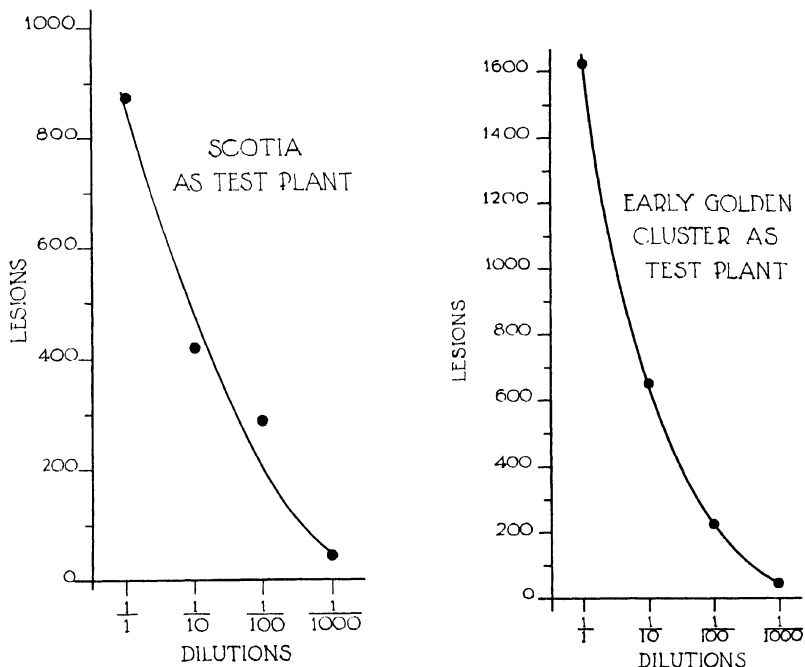
TEXT FIG. 2. Lesions produced on leaves of Early Golden Cluster beans following inoculation with tobacco mosaic virus. Leaf 1 was inoculated with an undiluted virus, leaf 2 with a 1 : 10 dilution, leaf 3 with a 1 : 100 dilution and leaf 4 with a 1 : 1000 dilution.

in this test had been previously inoculated with an undiluted virus it might be expected that all of them would retain a small amount of virus. However, considerably more virus would be expected in any leaves in which multiplication might have occurred. The relatively large number of lesions produced on leaves of *N. glutinosa* when inoculated with juice from leaves of Scotia and Early Golden Cluster is evidence that the virus had multiplied in leaves of these plants. The fact that all 11 plants inoculated with juice

from diseased leaves developed symptoms of tobacco mosaic, while only three of the 15 plants inoculated with juice from leaves on which no lesions occurred became infected, is significant and indicates that the virus had multiplied in the infected plants tested.

#### DILUTION EXPERIMENTS

If the number of lesions appearing on the leaves of inoculated plants can be correlated with the concentration of virus used as inoculum, it might be possible to use the number of lesions as a measure of virus concentration.



TEXT FIG. 3. *Phaseolus vulgaris* var. Early Golden Cluster as test plant. Figures 3 and 4 show the effect of diluting tobacco mosaic virus samples. In each case, the number shown represents the average number of lesions appearing for each leaf when eight leaves are rubbed with the virus sample tested.

TEXT FIG. 4. *Phaseolus vulgaris* var. Scotia as test plant.

In tests to determine whether such a correlation exists, plants of the varieties Scotia and Early Golden Cluster were used because the lesions appearing on the leaves of these varieties are numerous and large enough to be easily counted. Juice was extracted from mosaic tobacco plants, immediately diluted with water, and kept frozen until used for inoculation. After the first compound leaves had appeared, eight plants of each of the varieties

were inoculated by rubbing a given dilution of virus over the upper surfaces of the leaves. The dilutions of virus samples used in this test were, undiluted, 1 : 10, 1 : 100, and 1 : 1000. The result of the test is shown on leaves pictured in text figure 2. Lesions appeared on these plants the second day after inoculation but were not counted until the fifth day when they were somewhat larger. The number of lesions on each inoculated leaf is graphically shown in text figures 3 and 4. These figures show that the number of lesions decreases as the virus sample becomes more dilute. More extensive experiments are necessary to determine how well this

TABLE 1. *Susceptibility of Bean Varieties to Tobacco Mosaic \**

Variety Inoculated	No. of Plants In- oculated	No. of Plants Infected	No. of Plants In- oculated with Water	No. of Plants not Inoculated
Group 1:				
1. Early Golden Cluster.....	8	All	4	4
2. Ideal Market.....	8	"	2	4
3. Scotia.....	8	"	4	4
4. Cut Short or Corn Hill.....	8	"	4	4
5. White Creaseback.....	6	"	4	4
6. Stringless Refugee.....	7	"	—	2
7. Hodson Long Pod.....	5	"	1	4
8. Keeney's Stringless Refugee.....	7	"	—	4
9. Refugee Green Pod.....	7	"	4	3
10. New Navy Robust.....	1	"	—	—
Group 2:				
11. Unrivald.....	8	7	—	1
12. Improved Round Pod Valentine.....	8	1	4	4
13. Great Northern.....	8	4	2	4
14. Refugee Extra Early.....	7	4	—	5
15. Full Measure.....	6	1	3	—
Group 3:				
16. Bountiful.....	7	0	3	—
17. Dwarf Horticultural.....	7	0	4	3
18. Early Red Valentine.....	8	0	2	—
19. King of the Earlies.....	6	0	2	2
20. Longfellow.....	7	0	4	4
21. Masterpiece.....	8	0	—	4
22. Tendergreen.....	7	0	4	4
23. Henderson Stringless.....	1	0	4	—
24. White Marrow.....	7	0	3	—
25. White Kidney.....	3	0	3	—
26. Red Kidney.....	8	0	4	—
27. Giant Stringless.....	8	0	4	3
28. Tennessee Green Pod.....	3	0	—	1
29. Sutton's Masterpiece.....	7	0	3	4
30. Low's Champion or Red Cranberry.....	7	0	3	2
31. Improved Black Wax.....	8	0	4	3
32. Golden Age.....	7	0	1	1
33. Sure Crop Stringless.....	4	0	3	—
34. Wardell's Kidney.....	4	0	3	1
35. Burpee's New Kidney.....	1	0	1	—
36. Monster Stringless.....	3	0	2	—
37. Round Pod Kidney.....	12	0	—	1
38. Prolific German Black Wax.....	4	0	1	—
39. Violet Wax.....	1	0	—	1

TABLE I.—*Continued*

Variety Inoculated	No. of Plants In- oculated	No. of Plants Infected	No. of Plants In- oculated with Water	No. of Plants not Inoculated
40. New White Stringless.....	4	0	—	—
41. Improved Golden.....	3	0	4	—
42. Pencil Pod.....	7	0	4	4
43. Davis Kidney.....	4	0	—	—
44. Webber Wax.....	4	0	1	—
45. Currie's Golden.....	7	0	3	4
46. Currie's Rust Proof Black Wax.....	6	0	2	—
47. Davis White Wax.....	7	0	2	1
48. Dwarf Golden Carmine.....	4	0	3	1
49. Improved Rust Proof Golden.....	4	0	2	1
50. Horticultural.....	8	0	4	—
51. Horticultural Cranberry.....	8	0	4	4
52. Lazy Wife.....	4	0	2	—
53. Scarlet Runner.....	8	0	4	4
54. Burger's Stringless.....	5	0	2	2
55. White Dutch Runner.....	4	0	2	—
56. Kentucky Wonder.....	16	0	6	5
57. Fordhook.....	4	0	1	—
58. Henderson's Bush Lima.....	5	0	3	—
59. Henderson's Early Giant.....	4	0	1	—
60. New Wonder.....	2	0	1	—
61. Burpee's Improved.....	2	0	1	—
62. Early Leviathan.....	3	0	2	—
63. Large White Lima.....	3	0	1	—
64. Henderson's New Ideal.....	1	0	—	—
65. Large Lima.....	1	0	—	—
66. King of the Garden.....	4	0	3	—
67. Extra Early Jersey.....	4	0	2	—
68. Ford's Mammoth.....	3	0	1	—
69. Carpinteria.....	7	0	2	—
70. Large Green Seeded Lima.....	2	0	3	—
71. Siebert's Early.....	6	0	1	2
72. Ideal Lima.....	3	0	1	—
73. Dreer's Improved Pole.....	5	0	1	1
74. Early Jersey.....	1	0	1	—
75. Sieva.....	3	0	2	—
76. Dreer's Bush.....	5	0	2	—
77. Dreer's Wonder Bush.....	7	0	1	—

\* This table shows the bean varieties on which local necrotic lesions are produced when the leaves are rubbed with virus of tobacco mosaic. All check plants shown in the last two columns remained free of lesions.

correlation holds under various conditions. The tests indicate that it will be possible to work out a standard measure of virus concentration using plants of one or more of the susceptible bean varieties.

#### DISCUSSION

The fact that bean plants are easily and quickly grown from seed makes them desirable for use in measuring tobacco mosaic virus concentration. Two or three months are required to grow *N. glutinosa* plants to the stage at which they are suitable for inoculation. Bean plants, however, require



only eight or ten days to reach this stage. Obviously, the saving in time is considerable.

Many varieties of beans have proven to be susceptible to the virus of bean mosaic. Plants affected with this mosaic have a distinct mottled appearance quite different from the appearance of plants on which necrotic

TABLE 2. *Varietal Susceptibility* \*

Variety Inoculated	No. of Plants Inoculated	No. of Plants Infected	No. of Plants Inoculated with Water	No. of Plants Inoculated with Healthy Juice
1. Early Golden Cluster.....	11	All	4	4
2. Ideal Market.....	12	"	4	4
3. Scotia.....	12	"	4	4
4. Cut Short or Corn Hill.....	12	"	4	3
5. White Creaseback.....	8	"	3	2
6. Stringless Refugee.....	9	"	3	4
7. Hodson Long Pod.....	12	"	3	3
8. Keeney's Stringless Refugee.....	7	"	3	3
9. Refugee Green Pod.....	7	"	4	3
10. New Navy Robust.....	7	"	1	4
11. Unrivald.....	8	None	3	3
12. Improved Round Pod Valentine.....	9	1	4	4
13. Great Northern.....	11	All	4	1
14. Refugee Extra Early.....	8	2	4	4
15. Full Measure.....	10	3	4	4

\* The table confirms the results of groups 1 and 2 of table 1. Check plants inoculated with water (column 3) and plants inoculated with juice from healthy tobacco (column 4) remained free from lesions.

TABLE 3. *Transfer of Tobacco Mosaic Virus from Lesions on Bean Leaves Back to Tobacco Plants* \*

Variety Used as a Source of Inoculum	No. of <i>N. tabacum</i> Plants Inoculated	No. of <i>N. tabacum</i> Plants Infected	No. Lesions on <i>N. glutinosa</i>
Many lesions following inoculation:			
1. Ideal Market.....	1	1	1
2. Extra Early Refugee.....	2	2	6
3. Great Northern.....	1	1	7
4. Scotia.....	1	1	49
5. Early Golden Cluster.....	1	1	35
No lesions following inoculation:			
6. White Dutch Runner.....	2	0	0
7. Horticultural Cranberry.....	2	2	0
8. Prolific German Black Wax.....	2	1	0
9. Monster Stringless.....	2	0	0
10. Longfellow.....	2	0	0

\* The table shows the number of *N. tabacum* plants infected and the number of lesions developed on single plants of *N. glutinosa* when their leaves are rubbed with juice from inoculated bean leaves. Varieties 1-5 are those on the leaves of which lesions were produced following inoculation with mosaic virus. Varieties 6-10 are those on the leaves of which no lesions occurred following inoculation.

lesions are produced as a result of inoculation with tobacco mosaic virus. Reddick and Stewart (2, 3) tested a large number of varieties for resistance to bean mosaic. It is interesting to note that there is no correlation between susceptibility to tobacco mosaic and susceptibility to bean mosaic. Some varieties, such as White Creaseback, susceptible to tobacco mosaic, are immune to bean mosaic. Other varieties, such as Red Kidney, which are immune to tobacco mosaic, are susceptible to bean mosaic. Still other varieties, such as White Marrow, are resistant to both.

Local lesions caused by the virus of the ring spot disease of tobacco have been reported by Wingard (4) on kidney bean and on lima bean. Neither of these developed lesions when plants were inoculated with virus of tobacco mosaic. Plants of some of the varieties of beans susceptible to infection with tobacco mosaic virus have been inoculated with a sample of ring spot virus. The lesions produced by this virus are similar to but distinctly different from those caused by tobacco mosaic on the same varieties.

#### SUMMARY

1. Certain varieties of the common garden bean, *Phaseolus vulgaris*, develop local necrotic lesions when juice containing virus of the ordinary field type of tobacco mosaic is rubbed onto the upper surfaces of the leaves.
2. More virus was recovered from bean leaves on which lesions occurred than from similarly inoculated leaves of immune varieties on which no lesions occurred. This indicates that multiplication of virus took place in leaves of susceptible varieties.
3. Fewer lesions appear on leaves inoculated with dilute samples of virus than on leaves inoculated with concentrated samples. Curves are presented which indicate the possibility of using the number of lesions on susceptible varieties of beans as a measure of virus concentration.

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3. ——. Additional varieties of beans susceptible to mosaic. Phytopath. 9: 149-152. 1919.
4. Wingard, S. A. Hosts and symptoms of ring spot, a virus disease of plants. Jour. Agr. Res. 37: 127-153. 1928.

## AN IODIMETRIC METHOD FOR DETERMINING OXIDASE ACTIVITY \*

JOHN D. GUTHRIE <sup>1</sup>

The usual methods for estimating oxidase activity depend on either the production of a colored substance,<sup>2</sup> or the measurement of the volume of oxygen absorbed.<sup>3</sup> Recently an electrometric method has been suggested.<sup>4</sup> Colorimetric methods are often inapplicable on account of pigments or turbidity in the extracts to be tested. Methods measuring the oxygen uptake require special apparatus. The method to be described here requires no unusual equipment, is easy to use and reasonably accurate. With it as many as sixteen determinations have been made at the same time.

In a previous paper,<sup>5</sup> it was noted that potato juice contains a substance or substances that may be titrated with iodine in acid solution (trichloroacetic acid) and that this titration decreases on exposure to air. Ordinarily five cc. of juice reduces about 0.5 cc. of *N*/100 iodine. However, juice from one lot of potatoes was found to reduce 2.0 cc. of *N*/100 iodine. It was thought that this might be due to a low content of oxidase, the substance responsible for the iodine reaction not being oxidized in the process of extraction for this reason. In order to test this point, juice of this lot of potatoes was boiled and filtered. It still reduced 2.0 cc. of *N*/100 iodine. To 50-cc. portions of this boiled, filtered juice, 1 cc. of fresh juice from the same lot of potatoes and also 1 cc. from a lot giving the usual iodine titration were added. On exposing these mixtures to air in a thin layer, the iodine titration of the one containing the juice suspected of being low in oxidase had decreased 0.8 cc. after forty-five minutes, while the titration of the one containing juice of the other lot had decreased 1.4 cc. A water blank decreased 0.2 cc.

These results suggested that if a substance could be found that would reduce iodine in acid solution and which would also be oxidized by the air in the presence of potato juice, a convenient method would be available for determining oxidase. Cysteine was first tried. Its oxidation is catalyzed by potato juice, but the reaction proved to be autocatalytic and therefore unsuitable for the purpose. The autocatalytic nature of the oxidation of cysteine has been previously noted.<sup>6</sup>

\* Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 6.

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

<sup>2</sup> J. A. Dye, *Proc. Soc. Exptl. Biol. Med.*, **24**, 640-642 (1927).

<sup>3</sup> H. H. Bunzel, *THIS JOURNAL*, **34**, 303-316 (1912).

<sup>4</sup> A. E. Stearn and A. A. Day, *J. Biol. Chem.*, **85**, 299-306 (1929).

<sup>5</sup> F. E. Denny, L. P. Miller and J. D. Guthrie, *Am. J. Botany*, **17**, 483-509 (1930).

<sup>6</sup> M. Dixon and H. E. Tunnicliffe, *Proc. Roy. Soc. (London)*, **94B**, 266-297 (1923).

Szent-Györgyi<sup>7</sup> has noted the iodine reaction of plant juices and has isolated a substance that reduces iodine in acid solution from the adrenal cortex, orange and cabbage. He finds it to be a hexuronic acid. For this reason glucose that had been warmed with dilute sodium hydroxide was tried, since this product is known to contain a great variety of carbohydrate derivatives and it was thought that some of these might reduce iodine in acid solution. It was found that the iodine titration was quite large. Tests showed that it decreased on exposure to air and that this oxidation was catalyzed by potato juice.

**Preliminary Work.**—During the first part of the work, the reaction was carried out in liter beakers, 25 cc. of the reacting mixture being exposed in a thin layer in the bottom. At intervals, 5-cc. aliquots were drawn, 10 cc. of 10% trichloro-acetic acid was added and titrated with *N*/100 iodine, using starch as an indicator. The first difficulty encountered was a high blank, but it was found that clearing the substrate with decolorizing charcoal obviated this. It was also found that the conditions for aeration were unsatisfactory, since the rate of oxidation ceased to be directly proportional to the concentration of enzyme when more than 1 cc. of potato juice was used. Carrying out the reaction in aeration tubes corrected this difficulty. This improvement necessitated the use of a foam breaker. Capryl alcohol was tried, but all samples available interfered with the end-point, probably due to some impurity. It also had a slight retarding effect. Amyl alcohol, while not affecting the end-point, was decidedly injurious to the enzyme. Paraffin oil, although not so efficient a foam breaker as the higher alcohols, was finally chosen. In order to test the effect of foaming, the addition of digitonin was tried. It greatly increased foaming but did not affect the results. As an additional improvement it was found that more accurate results could be obtained by adding a known quantity of iodine, allowing to stand and then titrating the excess with thiosulfate.

**Preparation of Substrate.**—Dissolve 40 g. of glucose in 400 cc. of *N* sodium hydroxide, place it in a 500-cc. Florence flask and immerse in a water-bath at 80° for fifteen minutes. Remove and neutralize at once by adding 10 cc. of 85% phosphoric acid. Add 25 g. of decolorizing charcoal (Norit A was used) and allow to stand overnight. Filter and add 25 g. of decolorizing charcoal to the filtrate. Allow to stand for fifteen minutes and filter. Dilute a small portion of the filtrate about one to five and determine the *PH* value. If it is not close to *PH* 6.5, adjust to this *PH* with *N* sodium hydroxide or *N* hydrochloric acid. The addition of 2 cc. of either to 100 cc. of the filtrate shifts the acidity about 0.1 *PH*. The iodine value for 25 cc. should be equal to about 60 cc. of *N*/50. Before using, dilute the filtrate with an equal volume of water.

**The Method.**—Pipet 25-cc. portions of the diluted substrate into Van Slyke-Cullen<sup>8</sup> aeration tubes. Add 2 cc. of the juice or extract containing the enzyme. For each determination run a blank, using 2 cc. of the boiled, filtered juice or extract. Add five drops of paraffin oil to each tube and aerate for one hour. Wash into 300-cc. Erlenmeyer flasks containing 25 cc. of 10% trichloro-acetic acid, adding in all about 50 cc. of water. Add

<sup>7</sup> Szent-Györgyi, *Biochem. J.*, **22**, 1387-1409 (1928).

<sup>8</sup> D. D. Van Slyke and G. E. Cullen, *J. Biol. Chem.*, **19**, 211-218 (1914).

50 cc. of  $N/50$  iodine in  $N/10$  potassium iodide and allow to stand for thirty minutes. Titrate with  $N/100$  sodium thiosulfate, using 1 cc. of 1% starch paste as an indicator. Titrate the blank first and the determination immediately afterward. The difference between these titrations is a measure of the oxidase activity of the sample.

**Accuracy of the Method.**—In order to test how nearly results could be duplicated, twelve determinations were made on the same lot of potato juice. The average value was 6.15 cc. with an average error of  $\pm 0.15$  cc. To see how nearly the substrate could be duplicated, four batches were prepared and used with the same potato juice. The average value was 6.50 cc. with an average error of  $\pm 0.2$  cc. This was repeated with four other batches of substrate and other potato juice. The average value was 6.8 cc. with an average error of  $\pm 0.15$  cc.

**Choice of  $P_H$  Value.**—Acidity of the reacting medium greatly affects the activity of enzymes. Therefore, a series of determinations was made at various  $P_H$  values. These were obtained by the addition of  $N$  sodium hydroxide or  $N$  hydrochloric acid to the substrate. The quinhydrone electrode was used. Some difficulty was experienced, probably due to the interference of reducing substances. On the concentrated substrate values that were too alkaline were obtained. More acid values, which are believed to represent nearly the true  $P_H$ , were obtained by diluting with about five volumes of water and using a large amount of quinhydrone. This effect is probably brought about by diluting the interfering substances and thereby minimizing their effect. Several experiments were made to determine the effect of the  $P_H$  value of the substrate. A curve showing one of these, which is typical of the others, is shown in Fig. 1. Between  $P_H$  6.0 and  $P_H$  7.0 the oxidase activity

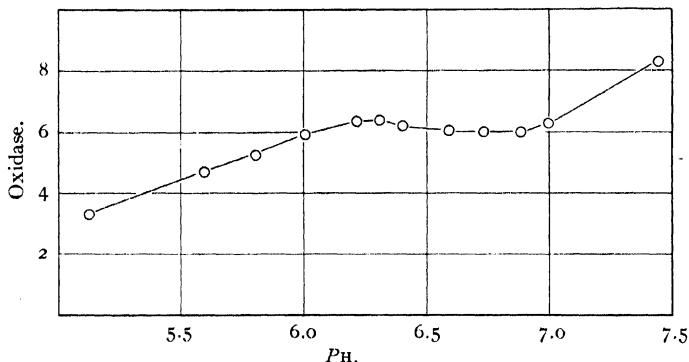


FIG. 1. Showing the effect of hydrogen-ion concentration on the oxidase activity of potato juice. Aerated for one hour; two cc. of juice used.

is not greatly affected. Therefore,  $P_H$  6.5 was chosen as the best for carrying out the determinations. Bunzel<sup>9</sup> has investigated the effect of hydrogen-ion concentration on oxidase activity and recommends approximate neutrality for making the determinations. His data, however, are insufficient to show the exact form of the  $P_H$ -oxidase curve, especially between  $P_H$  6.0 and 7.0.

**Effect of Concentration of Enzyme and Time of Aeration.**—In order to test the effect of the concentration of enzyme and decide on the time of aeration, experiments were made using one, two, three and four cc. of potato juice and aerating for different periods. In the first experiments which were

<sup>9</sup> H. H. Bunzel, *ibid.*, 28, 315-333 (1916).

aerated for two hours the oxidase activity was not linear with the concentration, but curved upward. This tendency was negligible when the aeration was only for one hour. It was found that the reason for this was the protective action of the juice, the enzyme in the tubes containing the larger amounts of juice being better protected. This protective action is present in the boiled, filtered juice. Therefore, an experiment was made in which the tube with 1 cc. of fresh juice contained also 3 cc. of boiled juice, the tube with 2 cc. of fresh juice, 2 cc. of boiled juice, and so on. Thus each tube contained 4 cc. of juice, but different amounts of enzyme in each. The results are shown in Fig. 2. It will be seen that the oxidase activity

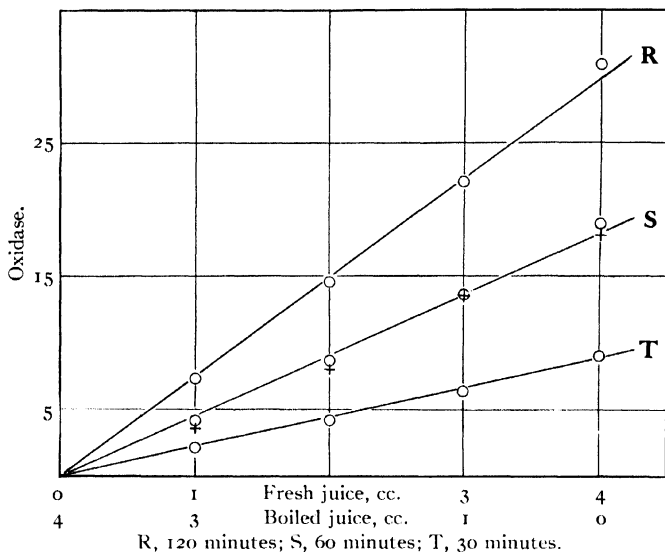


FIG. 2. Showing the effect of enzyme concentration and time of aeration. Circles determined with usual substrate concentration, crosses with half this concentration.

is almost linear with the concentration of enzyme even with two hours' aerating. Up to one hour the reaction is linear with time. In the second hour the reaction goes more slowly, showing that some enzyme is being destroyed. Therefore, one hour has been chosen for the time of aeration. Points are also given for the one-hour aeration curve in which half the usual concentration of substrate was used. Very little difference is noted, showing that in this range substrate concentration is not an important factor.

**Results with Other Plants.**—In order to see if the methods could be used on other materials besides potato juice, several other plant juices were tried. The results are shown in Table I. Qualitative tests were also made with the indophenol reagent.<sup>10</sup> The quantitative results correlate well with the qualitative.

<sup>10</sup> H. M. Vernon, *J. Physiol.*, **42**, 402-432 (1911).

TABLE I

APPLICATION OF METHOD TO VARIOUS PLANTS. TWO CC. OF JUICE USED UNLESS OTHERWISE NOTED

Plant	Iodimetric oxidase		Indophenol oxidase
Onion (bulb)	0.6	0.4	—
Turnip (root)	0.3	0.3	—
Beet (root)	7.7	8.3	++
Beet (leaves)	7.6	7.7	++
Carrot (root)	2.3	2.5	+
Apple (fruit)	2.6	2.2	++
Tomato (leaves) 0.5 cc.	11.8		+++++
Tomato (stems) 0.5 cc.	1.3		++
Tobacco (healthy leaves) 0.5 cc.	4.4		+++
Tobacco (mosaic leaves) 0.5 cc.	12.8		+++++

### Summary

An iodimetric method is given for the estimation of oxidase activity. Oxidase activity as measured by the method is a linear function of the enzyme concentration. The effect of hydrogen-ion concentration on potato oxidase has been studied. The method is applicable to a variety of plants.

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## LOCAL AND SYSTEMIC INCREASE OF TOBACCO MOSAIC VIRUS<sup>1</sup>

FRANCIS O. HOLMES

### INTRODUCTION

A number of investigators have studied the increase and spread of viruses in plants. Miss Purdy (7) described the production of mosaic virus in full-grown detached leaves of *Nicotiana tabacum*. Severin (8) determined the time required for a minimum amount of curly top virus to leave the inoculated area and pass a given distance in the sugar beet. McCubbin and Smith (5, 6) and Böning (1) made similar observations for mosaic virus in tomato and tobacco. Storey (9) made measurements of the same sort for streak virus in maize. Holmes (2) showed the time required for the production of mosaic virus in various concentrations in *N. tabacum*.

The development of a method of measuring virus concentration (3), by means of which large numbers of measurements may be made with comparative ease, has made it possible to secure further information in the case of tobacco mosaic disease. The purpose of this paper is to record the time required for the appearance of measurable amounts of tobacco mosaic virus at different distances from the site of inoculation in the inoculated leaf, in other leaves, in root and top, and in the stem of the plant; and to compare the subsequent increase of virus concentration in these parts with the increase at the site of inoculation.

### METHOD

Plants of *Nicotiana tabacum* var. Turkish were inoculated with tobacco mosaic virus on restricted areas, as on single quarters of large leaves, to allow a study of the time elapsing before the appearance of measurable concentrations of virus at points distant from the site of inoculation, and of the rate of increase in concentration of virus at these points and at the site of inoculation. Samples of parts of these plants were collected at intervals, wrapped separately in small squares of cheesecloth, and pounded to express their juices. The juices were tested by a method described briefly in an earlier publication (3). This method takes advantage of the fact that *Nicotiana glutinosa* reacts to inoculation with tobacco mosaic virus by forming necrotic spots at the site of inoculation. Large numbers of these

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.



necrotic local lesions appear on the leaves of the *N. glutinosa* plants when they are inoculated with undiluted juice samples containing virus, and small numbers when the juice containing the virus has been diluted. To produce these lesions for use in estimating the relative concentrations of virus samples the following procedure has been uniformly used.

Plants of *N. glutinosa* were grown in four-inch pots until blossom buds began to form. The plants were trimmed by removing their growing tips and small upper leaves, as well as some old leaves near the base, leaving on each plant five large green leaves on a sturdy stem. Virus samples were prepared by pounding the pieces of tissue wrapped in cheesecloth. The moist cheesecloth was rubbed uniformly over the entire upper surfaces of the five leaves of the plant of *N. glutinosa*. The inoculated plant was then washed with water. It has been found that washing the plant with water after the inoculation never decreases the number of successful inoculations, and may increase the number, especially if the fluid sample containing the virus to be measured also contains some substances harmful to the tissues of the inoculated plant.

The lesions resulting from the inoculation appear after varying intervals of time. At 24° C. the inoculated leaves still appear green and normal after thirty hours; yet six hours later, only one and a half days after the inoculation, the first lesions are to be seen. At the end of forty-eight hours one half of the total number of lesions are visible, and after four days nearly all have appeared. Final results may be recorded on the fifth day at this temperature.

The purpose of this research was to determine the time of appearance of quantities of virus large enough to be easily measured at given points in inoculated tobacco and tomato plants. The technique employed was not suitable for showing whether or not very small traces of virus might have been present at these locations earlier, and hence did not result in a determination of the rate of spread in any location. The cutting experiments of McCubbin and Smith (5, 6) and of Böning (1) bear more directly on this phase of the question of the distribution of virus. These investigators cut away the inoculated portions and some inches of healthy stem at intervals to test whether or not virus had passed definite distances. The experiments reported in this paper show the presence of virus only after it has begun to increase in concentration in a given location. Successive measurements in each location tend to confirm each other and to indicate whether an increase in concentration occurs in each portion studied.

#### COMPARATIVE SUSCEPTIBILITIES OF *N. GLUTINOSA* AND *N. TABACUM*

In spite of the fact that only relatively large and increasing amounts of virus have been considered significant in the experiments reported in this paper, some criticism of the method of measurement might well be made if it were not first shown that the plant used for the measurements is reasonably

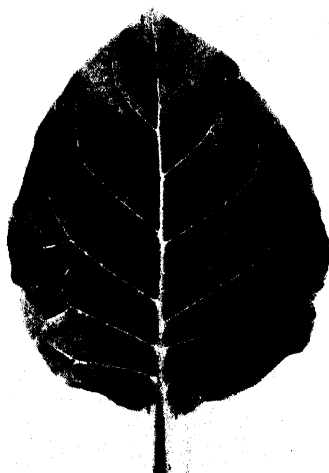
sensitive to inoculation. Since *N. tabacum* has been most commonly used for such measurements, the practical question is whether *N. glutinosa* is sufficiently susceptible to become infected by the rubbing method used in these experiments when the sample is so highly diluted that the presence of virus in it can be detected only with difficulty by means of *N. tabacum* with the same or other methods of inoculation.

An experiment was conducted to show the relations between the method used in these experiments and other methods. An estimate was made of the amount of water required to bring a frozen sample of virus to a strength sufficient to give an average of approximately ten lesions for each hundred leaves of *N. glutinosa* inoculated by rubbing. This dilution was one part of undiluted extract in one hundred and twenty-five thousand parts of water. Such a dilution was used to inoculate one set of *N. glutinosa* by rubbing, and four sets of *N. tabacum* var. Turkish by rubbing and by introducing fluid containing the virus into wounds made by scratching, crushing, and stabbing the leaves as described below. Thirty plants were used in each set. In the *N. glutinosa* set, which was inoculated by rubbing, twelve plants remained healthy, thirteen plants showed one lesion each, and five plants showed two lesions each. These two lesions were on different leaves of the plants in four of the five cases. Thus in this set eighteen plants in all produced one or more lesions. In the *N. tabacum* set, with plants of the same size rubbed with the same number of strokes with the same dilute virus sample, nineteen plants became diseased, and eleven plants remained healthy. Of the thirty *N. tabacum* plants scratched through the same sample of virus with twenty-five scratches each, only one plant became diseased. The virus used was so dilute (1 : 125,000) that with the small wounded area supplied by the scratches this result is not surprising. Similar results have been described previously (4) in comparing rubbing and scratching as methods of inoculation in *N. rustica*. Of the thirty *N. tabacum* plants inoculated by wetting the young leaves at the top of the plant and crushing these leaves with the fingers, seven became diseased and twenty-three remained healthy. Of the thirty *N. tabacum* plants punctured twenty-four times each in stem and petiole through a small wad of absorbent cotton wet with the same dilute virus, one plant became diseased and twenty-nine remained healthy. One hundred and fifty control plants, not inoculated but placed between the rows of the inoculated plants, all remained healthy during the course of the experiment. These tests indicate that rubbing is far more effective for inoculating *N. tabacum* than scratching, stabbing, or crushing, and that the difference of susceptibility of *N. tabacum* and *N. glutinosa* to rubbing inoculations with highly diluted virus samples is very slight. The *N. glutinosa* plants offer the advantages of distinguishing between single and multiple infections, of giving measurements over the wide range from undiluted extracts to dilutions of 1 : 1,000,000 without change of technique, and of reducing the probab-

ity of accidental transmission in the greenhouse, by reason of the very low virus content of *N. glutinosa* when infected.

#### SPREAD OF VIRUS WITHIN THE INOCULATED LEAF

The use of *N. glutinosa* for measuring the concentration of tobacco mosaic virus, with the method described above, made it possible to detect virus quantitatively even in small samples of tissue, and to repeat measurements frequently. It seemed possible, therefore, to attack the problem of the movement of virus from the inoculated portion of a leaf to other parts of the leaf, and to other parts of the plant. Quantitative measurements have not been reported previously to indicate whether or not virus spreads at once throughout the tissues of an inoculated leaf, nor has it been shown whether a large increase of virus occurs near the site of inoculation before it multiplies at the top of the plant where mottling appears.



TEXT FIG. 1. Leaf of *N. tabacum*, var. Turkish. Dotted lines show division into quarters, numbered as used in experiments on development of virus within the inoculated leaf, described in text and summarized in tables 1 and 2.

A number of plants of *N. tabacum* var. Turkish were studied in the following way to detect increase and spread of virus within the inoculated leaf if it should occur. Leaves not more than eight inches from the bases of stems of fifteen-inch plants were inoculated by rubbing extract of mosaic plants on one quarter of each leaf, the other quarters of the leaf being untouched. Two series of such leaves were studied, the inoculation being in a basal quarter in one series, and in an apical quarter in the other.

In the first series the leaves were inoculated in a basal quarter, indicated by number 3 in text figure 1. Care was taken not to inoculate the quarters represented by numbers 1, 2, and 4 in the diagram. Samples were examined at intervals to determine the concentration of virus in each quarter, similar quarters from three leaves being grouped to secure a large quantity for convenient testing. The midvein was discarded in all cases. The results of this experiment are shown in the first four columns of table 1. Virus

TABLE 1. *Measurements of Virus in Portions of Inoculated Leaf and in Distant Tissues of Tobacco Plants. Tests Made at Intervals After Inoculation of Leaf in Basal Quarter, Corresponding to Quarter Number 3 in Figure 1*

Interval After Inoculation	Quarters of Inoculated Leaf				Distant Parts of Plant	
	Quarter No. 1, Apical	Quarter No. 2, Apical	Quarter No. 3, Inoculated	Quarter No. 4, Basal	Leaves of Top	Complete Root System
1 day . . . . .	1	0	0	0	0	0
3 days . . . . .	0	0	142	0	1	0
5 days . . . . .	0	0	434	0	7	2
7 days . . . . .	0	0	1254	0	10	42
10 days . . . . .	0	0	1663	0	493	243
14 days . . . . .	0	0	621	44	219	110
21 days . . . . .	355	31	1741	433	527	317
28 days . . . . .	1138	750	746	412	769	1169

Measurements expressed in terms of number of lesions resulting from inoculation of *Nicotiana glutinosa* plants with extracts of tissues to be tested.

appeared in quantity in the inoculated quarter first. After two weeks measurable amounts of virus appeared in the opposite basal quarter, and subsequently in the two apical quarters of the leaf. The results of this experiment furnished evidence that virus may reach a high concentration near the site of inoculation before measurable quantities occur in distant portions of the leaf, but that distant parts of the leaf eventually contain virus.

In the second series the leaves were inoculated in one of the apical quarters of each leaf, indicated by number 1 in text figure 1. The quarters numbered 2, 3, and 4 in the diagram remained uninoculated in this case. The results are shown in the first four columns of table 2. In this case also the virus in the inoculated quarter soon reached a high concentration. After two weeks virus appeared in the opposite apical quarter of the leaf, which was the nearest of the three uninoculated quarters, and later in the two more remote basal quarters of the leaf. This confirmed the results of the first series, indicating a quick development of virus locally in the inoculated quarter of the leaf, and later slow passage to the uninoculated quarters.

TABLE 2. *Measurements of Virus in Portions of Inoculated Leaf and in Distant Tissues of Tobacco Plants. Tests Made at Intervals After Inoculation of Leaf in Apical Quarter, Corresponding to Quarter Number 1 in Figure 1*

Interval After Inoculation	Quarters of Inoculated Leaf				Distant Parts of Plant	
	Quarter No. 1, Inoculated	Quarter No. 2, Apical	Quarter No. 3, Basal	Quarter No. 4, Basal	Leaves of Top	Complete Root System
1 day . . . . .	0	0	0	0	0	1
3 days . . . . .	129	0	0	0	0	0
5 days . . . . .	304	0	0	0	0	0
7 days . . . . .	639	0	1	0	0	0
10 days . . . . .	677	1	0	0	349	179
14 days . . . . .	1168	25	3	0	805	414
21 days . . . . .	3848	207	188	375	1581	1337

#### APPEARANCE OF MEASURABLE CONCENTRATIONS OF VIRUS IN ADJACENT LEAVES

Quantitative measurements were made of the increase of virus in leaves adjacent to the inoculated leaf. Forty plants fifteen inches in height were inoculated with undiluted juice freshly extracted from mosaic plants. The inoculum was introduced into a single leaf about seven inches above the base of each plant by rubbing the leaf with cloth moistened with the extract. The potency of the inoculum was such that each leaf would receive much more virus than would be necessary to insure infection. At intervals a plant was examined by taking samples from the following parts: (1) its growing top, including all leaves less than an inch and a half in length, (2) the third leaf above the one inoculated, (3) the second leaf above the one inoculated, (4) the first leaf above the one inoculated, (5) the originally inoculated leaf, (6) the first leaf below the one inoculated, (7) the second leaf below the one inoculated, (8) the third leaf below the one inoculated, and (9) the lowest living leaf of the plant. At the beginning of the experiment these samples constituted practically the whole foliage of the plants. Later growth introduced numbers of leaves between the growing tip and the third leaf above the one originally inoculated.

The results of this experiment are summarized in table 3. A number of small observations, mainly to the number of ten or less, occurred in the early part of the experiment. These lesions may have been caused by virus from other sources necessarily handled during the experiment, or by virus left on the inoculated leaf after washing, and on other leaves of the plant from contact with water used to remove the excess of the inoculum. If the original mosaic extract in an experiment is dilute, such residues are very small and may not be detected. In the case under consideration the inoculum was concentrated, and large increases in the number of lesions in tests from a given location were necessary to ensure a significant determination of an increase of virus within the tissues of a sample.

TABLE 3. *Measurements of Virus in Inoculated Leaf, and in Leaves Above and Below Point of Infection in Tobacco Plants; Tests Made at Intervals After Inoculation*

Time	Leaves at Top of Plant	Third Leaf Above Inocu- lation	Second Leaf Above Inocu- lation	First Leaf Above Inocu- lation	Inocu- lated Leaf	First Leaf Below Inocu- lation	Second Leaf Below Inocu- lation	Third Leaf Below Inocu- lation	Lowest Leaf of Plant
0 days.....	0	0	1	4	85	0	0	1	1
1 day.....	0	0	0	0	217	0	0	2	1
2 days.....	2	0	2	0	557	1	2	0	1
3 days.....	19	0	0	0	802	0	0	0	0
4 days.....	0	3	3	2	944	0	0	0	0
5 days.....	0	2	0	1	936	9	0	2	1
7 days.....	1	0	0	1	771	0	0	0	0
8 days.....	306	0	7	5	1408	0	2	0	6
9 days.....	2	6	0	1	1660	0	0	0	3
10 days.....	1125	1	0	0	1069	0	0	0	85
12 days.....	742	0	3	0	1530	1	1	3	162
14 days.....	1920	156	0	1	2496	6	12	274	133
16 days.....	1311	0	42	3	1205	5	45	175	164
18 days.....	2086	0	0	4	2340	63	69	261	58
21 days.....	763	184	73	20	1374	543	147	553	123
24 days.....	943	264	426	262	—*	—	—	—	—
28 days.....	1411	1563	667	653	—	—	—	—	—
35 days.....	1100	278	—	—	—	—	—	—	—

\* Dashes in table indicate that measurements were not made because of death of lower leaves.

As is shown by the table, significant increases of virus occurred in the inoculated leaf before virus appeared in measurable quantities in other portions of the plant. This furnished evidence, in addition to that given by the experiments on tissues within the inoculated leaf, that in the systemic mosaic disease of *N. tabacum* the first development of virus is at or near the site of inoculation, with no immediate detectable diffusion of virus to other parts of the plant. Later samples showed that virus appeared in the developing leaves at the tops of the plants about the eighth day, when symptoms were becoming visible there. Virus was still absent from the other leaves of the plants at this time, except in the case of the leaf originally inoculated. Subsequent samples showed an increase of virus in the lowest leaf on the tenth day, and eventually in all the leaves of the plants. The experiment was discontinued when the lower leaves had died off at the end of five weeks from the time of the original inoculation.

#### SPREAD OF VIRUS TO ROOT AND TOP OF PLANT

It was known from inoculation experiments that the roots of mosaic plants contained virus in quantities sufficient to allow transfer to healthy plants. It was not known whether this virus was concentrated or not. It was also of interest to know whether virus would appear in the tops of the plants before appearing in the roots, or whether the reverse would be true. Tests were therefore made of tops and roots of plants in which the movement of virus within the inoculated leaves was being studied.

The numbers of lesions resulting from the inoculation of test plants with juices from tops and roots are recorded in the fifth and sixth columns of tables 1 and 2, accompanying the results of inoculation of juices from portions of the inoculated leaves of the same plants. The samples from the tops contained the growing point and all upper leaves less than one and a half inches in length, together with the short portion of stem supporting these leaves. The root samples were cut free of stem material and washed thoroughly before being tested.

Both tables show that there was a large increase in virus concentration in the inoculated leaf before measurable quantities of virus were detected in tops or roots. Both tops and roots began to show virus at about the same time and the virus content increased at about the same rate in both locations. It has been believed generally that mosaic virus passes to the tops of plants before it goes to other parts. This belief was probably based on the early development of mottling in the top leaves. Although not accompanied by visible symptoms, movement of virus down the stem to the roots seemed to occur as early in these experiments as movement upwards. Further experiments on the spread of virus to roots and tops of inoculated plants are described in a later section.

#### CONCENTRATIONS OF VIRUS IN PETIOLE AND STEM

The distribution of virus in parts already mentioned was studied in relation to distribution in petioles and portions of stem. The results shown in table 4 confirmed earlier observations, and in addition showed that appearance of measurable quantities of virus in the petiole of the inoculated leaf and in the stem was much delayed in some cases.

The experiment was carried out on plants which were about ten inches high when inoculated. Virus was introduced into one quarter of a leaf about half way up the plant. Each day a representative plant was divided into seventeen portions: (1) all top leaves one and a half inches in length or shorter, and the stem supporting them, (2) the second leaf above the one inoculated, (3) the first leaf above the one inoculated, (4) the first leaf below the one inoculated, (5) the second leaf below the one inoculated, (6) all leaves below these, (7) one basal quarter of the inoculated leaf, (8) the other basal quarter of the inoculated leaf, (9) one apical quarter of the inoculated leaf, (10) the other apical quarter of the inoculated leaf, including the whole inoculated area, (11) the petiole of this inoculated leaf, through which the virus must pass to reach the stem, (12) one inch of stem nearest to the petiole of the inoculated leaf, (13) two inches of stem just above this, (14) a second two inches of stem above, (15) two inches of stem just below the one-inch portion, (16) a second two inches of stem below, and (17) the roots washed free of dirt and carefully separated from the stem.

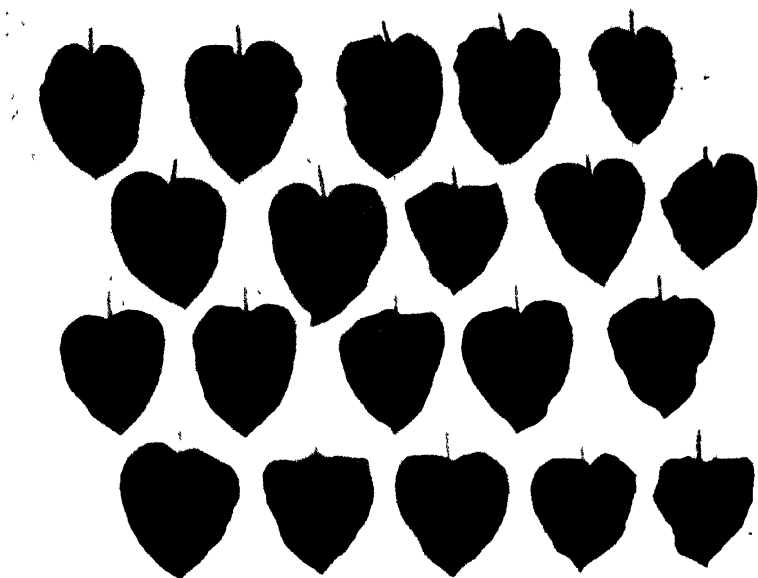
Virus appeared first at the site of inoculation, as it did in the experiments

TABLE 4. *Measurements of Virus in Leaves, Stem, and Root of Tobacco Plants at Various Intervals After First Introduction of Virus*

Days after Inoculation	0	1	2	3	4	5	7	8	9	10	11	12	14	15	16	17	18
All leaves at top of plant	0	0	0	0	0	0	0	672	2478	594	0	0	0	1007	352	715	724
Second leaf above inoculation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	2
First leaf above inoculation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
First leaf below inoculation	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	19	4
Second leaf below inoculation	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	18	77
All leaves below this	0	0	0	0	0	0	0	0	0	0	1	0	0	21	8	32	58
Left basal quarter of inoculated leaf	0	1	0	0	0	0	0	0	2	1	1	1	0	0	3	2	53
Right basal quarter of inoculated leaf	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	4	55
Left apical quarter of inoculated leaf	2	5	1	0	0	0	0	0	0	0	2	5	450	425	9	45	39
Inoculated quarter of inoculated leaf	28	47	114	142	784	728	2398	2089	2840	2789	2500	1462	1836	1213	1318	1199	833
Petiole of inoculated leaf	0	0	0	0	1	0	0	452	220	181	0	0	0	248	103	107	177
One inch of stem nearest inoculation	0	0	0	0	0	0	0	220	363	481	0	0	0	337	260	336	287
Two inches of stem above this	0	0	0	0	0	0	0	478	608	416	0	0	0	182	96	345	286
Second two inches above	—	0	0	0	0	0	0	546	772	323	0	2	0	310	299	369	562
Two inches of stem below one-inch sample	0	0	0	0	0	0	0	456	640	401	1	0	0	241	289	251	274
Second two inches of stem below	0	0	0	0	0	0	0	280	711	296	0	0	0	326	192	218	310
Roots	0	0	0	0	0	0	0	219	471	647	0	0	1	381	481	499	324



on spread of virus within inoculated leaves. At this point, as may be seen in table 4, a significant increase in virus concentration was reached by the second day, the first day reading being too small to distinguish from a chance variation from the amount used locally to inoculate the leaf. A very high concentration was reached in the inoculated quarter before any virus appeared in the three uninoculated quarters. Text figure 2 shows the



TEXT FIG. 2. Twenty leaves of the test plant, *N. glutinosa*, used to measure the concentration of virus in a leaf of *N. tabacum* eight days after its inoculation in an apical quarter. Top row: five leaves of the test plant, showing more than two thousand lesions resulting from inoculation with juice expressed from the originally inoculated quarter of the leaf of *N. tabacum*. Other three rows: leaves of three similar plants of *N. glutinosa*, showing no lesions as a result of inoculation with juice from the three uninoculated quarters of the *N. tabacum* leaf. See table 4, sample for eighth day.

tests of virus concentration on the eighth day, the top row of five leaves representing the test of the inoculated apical quarter, the row next below representing the opposite and uninoculated quarter, the next row one of the basal quarters, and the lowest row the other basal quarter. As may be seen by reference to table 4, the inoculated quarter had produced virus, giving more than two thousand lesions on the five leaves of the test plant, whereas the other three quarters showed no evidence of containing virus on this day.

Subsequently virus was found in increasing concentrations in the locations in which it had been found in earlier experiments. The second

quarter of the inoculated leaf to show a significant increase was the opposite apical quarter about the fourteenth day. Increases of virus were found later in the two basal quarters of the leaf. Before virus had been found in other quarters of the inoculated leaf than the inoculated quarter, the tops of some plants showed symptoms, consisting of rolling and vein clearing of the developing leaves about the eighth day, and subsequent mottling. On the eighth day, when several plants had produced such symptoms, one of them was taken as a representative sample, although a number of plants had not yet shown any symptoms. The plant with symptoms in the top leaves showed the first observed virus in that location. It also showed virus in the petiole of the inoculated leaf, in the five stem portions, and in the roots. On the ninth and tenth days plants with similar symptoms were used as representative samples with the same type of results. At this time some of the plants still showed no symptoms. On the eleventh, twelfth, and fourteenth days these green plants were used as samples. It was found that virus was present in them in high concentration at the site of the inoculation, and in the case of the fourteen-day sample it was found that the virus had spread across the midvein of the leaf to the opposite apical quarter, but no virus appeared in the petiole of the leaf inoculated, in the five stem portions, in the root, or in the top of any one of these plants. Subsequent samples were taken from the mottled plants which soon predominated in the set. These finally showed virus in the old leaves of the plant, both above and below the inoculated leaf, as may be seen upon examination of the results recorded in table 4.

This experiment furnished information not given by former tests. Its measurements showed that virus was always present at the top of the plant when symptoms were in evidence there. When virus was present in the top leaves it was also present in quantity in the whole stem and in the petiole of the leaf inoculated. The appearance of measurable concentrations of virus in tissues of the inoculated leaf not more than an inch or an inch and a half from the site of inoculation required approximately as long a time as the appearance of virus at the extremities of the plant. This suggests that the movement of virus in midvein, petiole, and stem is far more rapid than movement of virus within the leaf blade. For example, on the eighth day after inoculation virus had passed into all portions of stem and into the roots, but was not found in any quarter of the inoculated leaf except the originally inoculated quarter.

The regularity of increase in the inoculated area shown in this experiment was in marked contrast to the irregularity in time of appearance in measurable concentrations in the stem of the plant. This regular increase of virus near the point of inoculation may be useful in the study of the influence of external factors on the virus in the plant.

It seemed possible that although no gradient of virus concentration was found in this case, one might be found if the length of stem were greater.

The rate of increase in concentration in any one portion of stem is rapid as soon as measurable concentrations of virus appear there. If the movement of virus along the stem were relatively slow, a gradient of virus concentrations would be expected. The following experiment was conducted to furnish a better test of whether such a gradient could be found.

Ten tall specimens of Bonny Best tomato, each fifty-nine inches high, were inoculated in leaves about twenty-seven inches from the base. A section of stem six inches long near this point was discarded from each specimen, and frequent tests were made of eight six-inch sections, four above and four below this. The results of the experiment are shown in table 5. It does not seem possible to detect a significant gradient of virus

TABLE 5. *Measurements of Virus in Stem of Tomato Plants at Various Distances From Point of Inoculation*

Days After Inoculation	0-6 Inches, Lowest	6-12 Inches, Second	12-18 Inches, Third	18-24 Inches, Fourth	24-30 Inches, Discarded	30-36 Inches, Sixth	36-42 Inches, Seventh	42-48 Inches, Eighth	48-56 Inches, Highest
2	0	2	0	0	—	0	0	0	0
4	0	0	0	0	—	0	0	0	0
6	4	1	1	1	—	0	3	11	5
7	384	462	222	496	—	474	210	147	192
8	4	99	187	459	—	201	307	486	682
9	1388	1330	901	811	—	1093	627	654	724
11	417	592	489	528	—	513	519	543	711
Total lesions . . . . .	2197	2486	1800	2295	—	2281	1666	1841	2314

concentration in the data. The initiation of the systemic infection in the individual plants was probably irregular, the eighth and eleventh day samples being less advanced than earlier ones, but the failure of any one portion of the stem to contain large amounts of virus earlier than others is the significant result of the experiment. This is well shown by the totals at the bottom of the table, since the presence of virus over a much longer period in one section of a stem than in another would have caused an increase in the total of the observations for that location. Apparently when virus reached the stem it was distributed so quickly that measurements made as in this experiment were not sufficient to show whether it became concentrated in one part before appearing in all others. The increase of the virus in all portions of stem seemed to be approximately simultaneous, so far as the data of this experiment show.

For comparison it was considered a matter of interest to know how rapidly virus would reach a measurable concentration in a stem not receiving virus from attached leaves. Internodal portions of tomato stem were removed from stems of Bonny Best tomato, inoculated with undiluted virus by means of thirty pin pricks made with No. 00 insect pins, and held each in a sterilized test tube at 25° C. Four pieces of stem were crushed each day and the juices tested on *N. glutinosa* plants. The increase of

virus in the stems is shown by the results recorded in table 6. It will be seen that the small amount of virus introduced by the five pin pricks multiplied rapidly enough to produce a significantly larger number of lesions by the third day. The stems then produced virus at a rate comparable to that previously shown in leaves. Since the virus increased in

TABLE 6. *Measurements of Virus in Detached Tomato Stems at Intervals After Inoculation. The Stems Were Inoculated After Removal From Plants*

Time of Test	Four Comparable Series of Specimens				Average of Four Measurements
	Series 1	Series 2	Series 3	Series 4	
1 day . . . . .	1	7	1	0	2
2 days . . . . .	25	85	34	41	46
3 days . . . . .	465	280	115	87	237
4 days . . . . .	430	418	381	386	404
5 days . . . . .	490	433	565	342	458
7 days . . . . .	852	855	1183	590	870
10 days . . . . .	852	667	642	921	770
12 days . . . . .	1498	1762	2026	1037	1581
14 days . . . . .	1322	1038	775	1107	1060
16 days . . . . .	1597	1215	1036	1548	1349
20 days . . . . .	574	1037	1240	1070	980

concentration in these detached stems at about the same rate as shown in table 5 for the stems of plants with attached leaves containing virus, it seems probable that movement of virus in large quantities from these leaves to the stem need not be assumed in order to explain the increasing amounts of virus in the stem portions, although of course small quantities must escape to the stem to start the infection there.

#### FURTHER EXPERIMENTS WITH ROOTS AND TOPS OF PLANTS

In the experiment on the measurement of virus in tops and roots of plants inoculated in leaves about half way up the stems, and in the experiment on the measurement of virus in the stem after inoculation in leaves similarly situated, the evidence favored the view that the movement of virus was so rapid up and down the stem, that virus did not become noticeably concentrated in the portions of the stem near the point of inoculation before the time when the extremities of the plant also contained virus.

Another experiment with *Nicotiana tabacum* was made to determine whether the introduction of virus in an upper leaf would result in the appearance of virus in tops before roots; and to determine whether the introduction of virus in a lower leaf would result in the appearance of virus in roots before tops.

Plants were grown to a height of fifteen inches. In one series virus was introduced into a leaf not yet fully developed, about two inches below the top of each plant. The inoculated leaf, its petiole, the top one inch of the stem with its attached leaves, and the washed roots of plants were tested

at intervals, as indicated in table 7. Significantly increased amounts of virus appeared in the inoculated leaf on the second or third day. In this set of plants inoculated in a young leaf, virus appeared in the petiole of the inoculated leaf, in the top, and in the root on the fourth day. Symptoms of the systemic disease appeared on these plants on the sixth day.

TABLE 7. *Measurements of Virus in Different Parts of Tobacco Plants at Intervals After Inoculation in Young Leaves*

Time	Leaf Inoculated	Petiole of Leaf	Top of Plant	Roots of Plant
1 day.....	0	0	0	0
2 days.....	23	0	0	0
3 days.....	89	0	1	0
4 days.....	770	42	56	23
5 days.....	1270	355	1232	150
6 days.....	1414	108	575	227
7 days.....	1162	812	625	159

In the second series virus was introduced into an old leaf about two inches above the base of each plant. The two series were carried out simultaneously and on comparable plants. Samples were taken of the inoculated leaf, its petiole, the top one inch of the stem with its attached leaves, and the washed roots of plants. Results are recorded in table 8. Significantly increased concentrations of virus appeared in the inoculated leaf on the third day, but not in the petiole of the inoculated leaf, the tops, or the roots during the first twelve days after inoculation. The rate of increase of virus in these old leaves seemed to be approximately as great as that in the young leaves of the other series, but movement from the old leaf to other portions of the plant was delayed. On the sixteenth day two cases of the systemic disease appeared among the five remaining plants of this series. On the next day one more case appeared clearly, and of the remaining two plants one showed the very faintest detectable flecks of yellow in its new leaves. This doubtful case was examined by inoculating juices from its tissues as before. It showed virus in all the parts tested, thus giving evidence that even in this early stage of spread of virus from an old leaf located near the bottom of the plant, virus was in evidence in the distant top as well as in the nearby roots.

The roots showed an unusually high result in this case, which might have led to the conclusion that the inoculation of a leaf near the base of the plant allowed virus to reach the roots before reaching the top, but a more critical experiment showed that this was not true in general. The experiment was arranged to obtain adequate numbers of records at the critical moment, when for the first time virus was present in parts at a distance from the inoculated leaf. A set of plants like those used previously was inoculated as before in an old leaf about one inch from the base of each plant. Instead of testing the plants daily as in the previous experiment,

the plants were left in a greenhouse for ten days, when symptoms were visible in the tops of one-fourth of the plants. The plants with symptoms were discarded. Former experience indicated that most of the remainder of the set would have shown symptoms within a day or two if allowed to remain. Ten representative plants were tested at once in the hope of finding all of the early stages of spread of virus in them. The results are shown in table 9. Two plants gave no evidence of the presence of virus

TABLE 8. *Measurements of Virus in Different Parts of Tobacco Plants at Intervals After Inoculation in Old Leaves. Compare With Table 7*

Time	Leaf Inoculated	Petiole of Leaf	Top of Plant	Roots of Plant
1 day . . . . .	1	0	0	0
2 days . . . . .	6	0	0	0
3 days . . . . .	344	0	0	0
4 days . . . . .	431	0	0	1
5 days . . . . .	482	0	1	0
6 days . . . . .	1439	0	6	0
7 days . . . . .	904	0	5	1
8 days . . . . .	1050	0	0	0
9 days . . . . .	1310	0	0	0
10 days . . . . .	1067	0	0	0
12 days . . . . .	(Leaf dead, 474)	0	0	0
17 days . . . . .	(Leaf dead, 578)	359	517	806

TABLE 9. *Measurements of Virus in Different Parts of Tobacco Plants Ten Days After Inoculation in Old Leaves. None of the Plants Showed Symptoms When Tested*

Plant	Leaf Inoculated	Petiole of Leaf	Top of Plant	Roots of Plant
No. 1	1407	0	0	0
2	1035	0	0	0
3	887	1	0	7
4	1296	7	4	3
5	1305	46	92	4
6	648	42	81	70
7	583	155	50	8
8	1123	81	307	107
9	1144	177	976	26
10	794	522	697	700

except in their inoculated leaves, a third showed none in the top but such small concentrations in roots and petiole that the single negative result in the top did not constitute significant evidence against the presence of an equal amount there, and seven showed virus in all parts tested. This series of measurements gives more data than are given in table 8 on plants studied at the time of the first appearance of measurable amounts of virus at the extremities of the plant. When the results are considered as a whole they seem to furnish no evidence of appreciably earlier or later arrival of virus in roots than in tops.

A striking result of this set of experiments was the evidence that virus developing at approximately equal rates in young and old leaves, and coming to equally high concentrations in both, might spread from the young leaves to distant parts of the plants many days earlier than from the old leaves.

### DISCUSSION

Lesions resulting from the inoculation of plants of *N. glutinosa* increase in number as the concentrations of virus in the inoculated extracts increase, but the ratio between the number of lesions and the concentration of virus is a variable which decreases as both of these increase. That is, the number of lesions does not bear a constant relation to the concentration of the virus; just as the number of colonies produced upon pouring plates from a series of bacterial suspensions does not bear a constant relation to the number of bacteria in the original series of suspensions. Since it is customary to think of the number of bacteria in terms of the number of colonies produced on pouring plates, it may be permissible to record increases in mosaic virus concentration in various parts of the host plant in terms of the increase in the number of lesions produced upon the inoculation of extracts of samples of tissues.

It is possible to interpret the results of measurements of virus concentration expressed in terms of local lesions by means of previously established dilution tables, which show the relation between known water dilutions of virus and the number of lesions produced in *N. glutinosa*. A graph giving this information has been presented in an earlier paper (3). The samples measured in the work described in this paper were whole juice samples, and their interpretation by means of the results of inoculation with water dilutions may be open to criticism. Therefore, the number of lesions is presented in the tables of this paper without conversion into terms of relative virus concentration; but in such cases as this, in which the inoculated juices are not known to be seriously harmful to the cells of the test plant, such a conversion of the data might perhaps be made with propriety if it were desired to represent the relative concentrations of virus in different parts of host plants.

### SUMMARY

By means of measurements made with *Nicotiana glutinosa*, the concentrations of virus in portions of inoculated plants of *N. tabacum* var. Turkish were studied, and it was found that:

1. Mosaic virus developed to a high concentration near the site of inoculation in a leaf of *N. tabacum* before reaching measurable concentrations in other portions of the inoculated leaf or in other parts of the plant.

2. A slow spread of virus through the tissues of the inoculated leaf accompanied the increase in concentration near the site of inoculation, and appeared to be independent of the rapid spread which carried virus to distant parts of the plant. This local increase and slow spread of the virus constitute a local or primary phase of the disease.

3. The systemic or secondary phase of the disease was marked by the nearly simultaneous appearance of increasing quantities of virus in the petiole of the inoculated leaf, in all portions of the stem, in the developing top leaves of the plant, and in the root, with later invasion of old leaves.

4. In a series of plants all successfully inoculated in similar leaves at the same time, the local increase of virus within the tissues of the inoculated leaf blade occurred simultaneously in all plants; but systemic spread of virus, with its attendant mottling of developing leaves, occurred early in some individual plants and late in others.

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## SUCROSE AND STARCH CHANGES IN POTATOES TREATED WITH CHEMICALS THAT BREAK THE REST PERIOD <sup>1, 2</sup>

F. E. DENNY

According to previous experiments (2) the treatment of dormant potato tubers (*Solanum tuberosum* L.) with chemicals such as ethylene chlorhydrin and sodium thiocyanate greatly increased the sucrose content of the tissue. However, the comparisons were made merely between untreated potatoes and those treated with concentrations of chemical which were approximately optimum for inducing sprouting. No intermediate concentrations were tested. Consequently, it seemed that a study of the effect of suboptimal amounts of chemical would give more conclusive evidence regarding the reality of the sucrose increase, and, furthermore, would be of interest in showing the general relation between the concentration of chemical and the amount of the gain in sucrose.

In 1929 press-juices were obtained from potatoes that had been treated with amounts of chemicals increasing by steps from zero up to approximately the optimum. Ethylene chlorhydrin, sodium thiocyanate, and thiourea were included in the tests and the enzym activities of the juices were measured and reported upon in a previous paper (3). Samples for sugar analysis were also taken and the results here reported show that the relation between the concentration of chemical and the amount of sucrose found in the juice was very close, the sucrose increasing with the increase in chemical and giving in nearly all cases a series of sucrose readings corresponding to the concentrations of chemical used in treating the potatoes.

In the previous report (2) it was shown that sucrose increases were observed in lots that had been sampled only 48 hours after treatment, and it was stated that sampling would need to be started at an earlier period in order to learn the time of the initiation of the sucrose change. The experiments here reported upon show that the time at which increases in sucrose were observed varied with different experiments, and was as early as 24 hours and as late as 72 hours.

Finally, in the previous report it was shown (2, p. 332) that the starch content was found to have been decreased by the treatments, provided the tissue for the analysis was taken at the eyes of the tuber, but that if the balance of the tissue (seed-piece minus eye-tissue) was sampled the question

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of a difference between treated and check was somewhat in doubt. Small decreases in the treated tissue were observed but the differences were not consistent. In the present experiment samples of dried tissue from several of the series of treatments were available for analysis and attention was paid only to the not-at-eye tissue. The results were consistent in showing lower starch values in the treated tissues, but the differences were small. Furthermore, although the optimum concentration of chemical showed the largest decreases in starch, the relation between the concentration of chemical and the decrease in starch was not such as to give clear gradations of values such as were obtained with the sucrose tests.

### EXPERIMENTAL

*Experimental tubers.*—These were for the most part the same as those reported upon in the preceding article (3). In order to show the source of seed, time of harvesting, time of treatment, etc., table 1 has been prepared. These data apply to all of the lot numbers in the other tables in this paper.

TABLE 1. *Data on Tubers Used in Experiments*

Lot No.	Variety	Source of Tubers	Date Harvested	Date Treated	Date Sampled
137-141	Cobbler	Maryland	*	July 25	July 31
152-155	"	"	*	Aug. 1	Aug. 5
157-160	"	"	*	" 3	" 7
162-164	Bliss	Yonkers, Nepperhan Gardens	Aug. 6	" 8	" 12
167-171	"	" " "	" 6	" 9	" 14
177-181	"	" " "	" 6	" 13	" 17
182-185	"	" " "	" 6	" 16	" 20
187-190	"	" " "	" 6	" 19	see table 3
207-210	Cobbler	" " "	" 6	" 26	Aug. 31
211-212	"	" " "	" 6	" 26	see table 3
213-214	Bliss	" " "	" 6	" 26	" " "
221-223	Cobbler	" " "	" 6	Sept. 3	" " "
224-227	Bliss	Country Club Gardens	Sept. 3	" 11	Sept. 17
228-231	Cobbler	" " " "	" 3	" 11	" 16
232-235	Bliss	" " " "	" 3	" 14	" 19
236-239	Cobbler	" " " "	" 3	" 14	" 19
240-243	Bliss	" " " "	" 3	" 12	" 20
252-255	Cobbler	" " " "	" 3	" 18	" 23

\* About the middle of July, exact date not known.

*Chemical treatments.*—Three different chemicals were used: ethylene chlorhydrin ( $\text{ClCH}_2\text{CH}_2\text{OH}$ ), sodium thiocyanate ( $\text{NaSCN}$ ), and thiourea ( $\text{NH}_2\text{CSNH}_2$ ). Two different methods of treating with ethylene chlorhydrin were used: (1) Whole tubers were exposed for 24 hours to vapors of ethylene chlorhydrin in a closed container; the amounts of chlorhydrin (40 percent solution) used per liter of air space inside the container (without allowance for the volume of the tubers) ranged from 1.0 to 0.008 cc. (2) Seed-pieces obtained by cutting whole tubers into pieces about 25 grams each in weight were dipped into a dilute solution of ethylene chlorhydrin

and the dipped pieces were then placed in a container with a tight seal for a definite period, usually 24 hours, in some cases 16 hours, or even 8 hours; the concentration of the dipping solution ranged from 45 cc. to 5 cc. per liter of water.

The treatments with both sodium thiocyanate and thiourea consisted in soaking the cut tubers (not whole tubers) in the chemical solution for one hour and then planting without rinsing off the adhering chemical. The strength of the solution was varied from about one or two percent (10-20 grams per liter of water) to about one-fourth or one-eighth percent or even less. The exact amounts of chemicals used in each series are shown in column 2 of table 2.

In each series of concentrations used in each experiment the highest concentration is that which previous experience had shown to be approximately the optimum for these varieties and for this stage of dormancy. For the whole-tuber vapor treatments, one cc. per liter of air space for 24 hours is probably too high if the potatoes are to be cut and planted at once after treatment, but in the present experiments the treated tubers were not cut and planted at once after treatment but were allowed first to stand in air for five to seven days under which conditions this amount of chemical is not too high. For the thiourea treatments two percent is probably less effective than one percent for inducing good growth of sprouts after germination has started. Furthermore two percent thiourea induces the sprouting of too many sprouts per eye.

*Sampling.*—The methods of sampling the treated and check lots, and of squeezing the tissue to obtain press-juices are described in the previous article (2).

The time of sampling after treatment, as shown in the previous report (3), varied from four to seven days, and was chosen so as to allow the early stages in the initiation of germination to begin, but to avoid sampling when sprouting had taken place to any marked extent. In all cases, therefore, the samples were taken either before any sprouts were visible, or, in certain exceptional cases, when only a few of the seed-pieces in the treated lots showed evidence of the beginning of bud development.

*Chemical methods.*—For the sugar analyses a sample of the press-juice was dropped from a pipette into boiling 95 percent alcohol of sufficient volume to give a final concentration of 70 percent alcohol. When the analysis was started it was made up to volume in a flask and filtered. Aliquots of the alcoholic extract were placed in evaporating dishes, the alcohol removed by evaporation on a steam-bath and replaced gradually by water. The aqueous solution was made up to volume and aliquots taken for copper reduction before and after inversion. The inversion was carried out by acid in the cold (see 1, p. 95).

For the starch determinations samples of fresh tissue chopped to small pieces in a wooden bowl were dropped into boiling 95 percent alcohol and

allowed to boil for a few minutes. The dish was then placed on a steam-bath until nearly all of the alcohol was removed. The tissue was then placed before an electric fan and when it had become dry it was ground in a food-grinder and finally reduced to a fine powder in a power-driven grinder operating on the mortar-and-pestle principle. This powder was dried in an electric oven at 99° C. and portions weighed out for starch analysis. The acid-hydrolysis method (see 1, p. 95) was used, and the starch data, therefore, represent total acid-hydrolyzable substances calculated as starch. Some determinations were made with the Walton and Coe method (9) which eliminates the non-starch polysaccharids and thus gives a better figure for starch. But duplicate determinations on several samples of potato powder by the two methods showed that the Walton and Coe method gave a value uniformly about 0.9 of the acid-hydrolysis method indicating that non-starch polysaccharids formed only about one-tenth of the total formed by the acid-hydrolysis method. Because of the greater convenience of the acid method it was used exclusively in the rest of the tests.

*Sprouting response.*—The percentage sprouting for most of the lots is given in the preceding paper (3), p. 595. The percentages varied with different experiments, but a general statement is as follows: favorable concentrations of chemical induced 75 to 100 percent sprouting, the next lower concentration (which was about one-half to one-third of the optimum) induced 50 to 75 percent sprouting, while the values for the lower amounts of chemical were 20 to 50 percent; the check lots ranged from 0 to 20 percent. The important consideration is that the sprouting response formed a series corresponding to the concentrations of chemicals used in treating the potatoes.

## RESULTS

### Relation of Concentration of Chemical to the Development of Sucrose in the Press-juice of Potatoes

The sucrose contents of the juices obtained from the various lots which had been treated with different concentrations of chemicals are shown in table 2. In columns 2, 5, and 8 will be found the series of concentrations used in each test, the strength of the chemical being reduced step-wise, each lower concentration being some fraction, *e.g.*, one-half, one-fourth, one-third, etc., of the preceding value. In columns 3, 6, and 9 are shown the sucrose values in milligrams per five cc. of juice. It is seen that a gradation of sucrose values was obtained corresponding closely to the graded concentrations of chemicals used in treating the potatoes. Thus the favorable concentrations of ethylene chlorhydrin approximately doubled the sucrose content as compared with the check lot; the NaSCN-treated lots were about three times and the thiourea-treated lots were about 2.5 times as high in sucrose as the corresponding checks. When the concentration of chlorhydrin was reduced to one-third of the optimum value the

sucrose in the treated was then found to be about 1.5 times that of the check, and reducing the concentrations of NaSCN and thiourea to one-half strength caused the sucrose value to become about twice that of the check. Still further reductions in concentrations resulted in smaller differences between treated and checks, but even when the concentration was one-fourth or one-fifth the optimum gains of treated over checks were observed.

TABLE 2. *Effect of Chemical Treatment of Potatoes Upon the Sucrose in the Press-juice*

Ethylene Chlorhydrin Treatments*			Sodium Thiocyanate Treatments			Thiourea Treatments		
Lot No.	Conc. of Chem.	Sucrose mg. in 5 cc.	Lot No.	Conc. of Chem.	Sucrose mg. in 5 cc.	Lot No.	Conc. of Chem.	Sucrose mg. in 5 cc.
137	1.00 cc.	54.0	157	2.00%	31.2	152	2.00%	39.8
138	0.20 "	35.1	158	0.67%	35.6	153	0.67%	29.4
139	0.04 "	26.2	159	0.22%	22.4	154	0.22%	22.4
140	0.008 "	26.0	160	check	16.3	155	check	16.6
141	check	21.3						
240	0.50 cc.	56.2	167	1.000%	55.7	162	2.00%	57.3
241	0.17 "	54.8	168	0.500%	37.5	163	1.00%	43.5
242	0.06 "	42.4	169	0.250%	27.4	165	0.50%	40.1
243	check	38.2	170	0.125%	14.1	166	0.25%	34.6
			171	check	12.1	164	check	16.9
207	45 cc.	23.8	182	1.00%	60.0	177	1.000%	57.9
208	15 "	16.5	184	0.50%	25.4	178	0.400%	46.9
209	5 "	11.9	186	0.25%	25.2	179	0.160%	42.8
210	check	13.3	183	check	19.5	180	0.064%	37.1
			185	check	18.6	181	check	22.9
255	45 cc.	25.9	235	1.00%	71.1	224	1.00%	43.7
254	15 "	17.7	234	0.50%	54.0	225	0.50%	42.6
252	5 "	17.7	233	0.25%	41.1	226	0.25%	39.0
253	check	13.3	232	check	17.7	227	check	23.4
			239	1.00%	58.5			
			238	0.50%	37.4			
			237	0.25%	38.1			
			236	check	17.5			

\* Lots 137-141 and 240-243 treated by the whole tuber method and column 2 shows the number of cubic centimeters of 40 percent ethylene chlorhydrin used per liter of air space inside the container. Time of exposure to vapors, 24 hrs. Lots 207-210 and 252-255 treated by the cut-tuber dip-method and column 2 shows the number of cubic centimeters of 40 percent ethylene chlorhydrin added to one liter of water in preparing the solution into which the cut-tubers were dipped before storing in a closed container for 24 hrs.

NOTE: For varieties, source, dates of harvest, etc., corresponding to the various lot numbers, see table 1.

### Time Relation in the Sucrose Increase After Treatment

In this experiment samples of treated and check potatoes were removed at intervals of 24, 48, 72, 96, and 144 hours after treatment and the press-juices were analyzed for sugar. The results are shown in table 3, the time

TABLE 3. *Time Relation of Gain in Sucrose*

Time After End of Treatment	Sucrose, Milligrams per 5 cc. of Press-juice									
	Ethylene Chlorhydrin Treatment Lots. 211-212 *		Ethylene Chlorhydrin Treatment Lots. 213-214 *		Ethylene Chlorhydrin Treatment Lots. 187-188 †		Sodium Thiocyanate Treatment Lots. 189-190		Lot Nos. 221, 222, and 223	
	Treated	Check	Treated	Check	Treated	Check	Treated	Check	Sodium Thiocyanate Treated	Thiourea Treated
Start.....	—	17.7								
24 hrs.....	6.9	11.0	17.7	32.1	24.0	19.8	26.3	29.6	13.6	14.4
48 ".....	16.6	7.8	27.2	19.6	15.7	lost	31.2	14.5	18.3	7.2
72 ".....	27.8	10.5	27.2	16.0	16.8	14.1	34.4	17.4	21.2	19.1
96 ".....	48.4	17.7	35.3	16.0	28.3	18.2	52.1	13.0	49.3	45.1
144 ".....				16.8	25.2	15.9	54.1	13.6	lost	44.0
					52.9	22.4	50.0	16.9	38.5	38.5
										11.6
										6.4
										13.0
										17.4
										19.7
										10.7

\* Dip method, dipping solution 60 cc. per l., storage period 16 hours. (Check lots dipped in H<sub>2</sub>O.)† Dip method, dipping solution 30 cc. per l., storage period 24 hours. (Check lots dipped in H<sub>2</sub>O.)

Note: Check lots 190 and 223 soaked in water instead of sodium thiocyanate or thiourea solution.

(after the end of the treatment) at which the samples were removed being shown in column 1, and the sucrose values of treated and check juices at each sampling period being shown side by side in paired columns under the appropriate heading for the type of treatment applied. Increases in sucrose of the treated over the check were observed with the sodium thiocyanate treatments at the end of the 24 hour period. In general, however, it was not until the 48th hour that the increase became pronounced and in the case of the chlorhydrin lot Nos. 187-188 the increase did not occur until sometime between the 48th and 72d hour. It will be noted that the check lots usually lost sucrose during the first 24 hour period as a result perhaps of the high rate of respiration induced by cutting the tuber into pieces. With the thiocyanate treated lots, however, this loss in sucrose was not noted, the early and rapid production of sucrose probably more than compensating for the excessive loss by respiration. It is likely that the increased respiration accounts for the low values at the 24 hour period in the chlorhydrin-treated lots, since as shown by Smith (7) the chlorhydrin treatment promptly induces a high rate of respiration.

The maximum increase was reached by the NaSCN and thiourea lots at about the 72d or 96th hour at which times the sucrose of the treated lots was about three times as great as in the corresponding checks. The chlorhydrin treatments did not reach their maximum difference until about the 96th or 144th hour, and showed at that time sucrose values about twice those of the checks.

### **Effect Upon the Reducing Sugars**

Although in all cases shown in tables 2 and 3 the amount of reducing sugar was determined it seemed unnecessary to present the reducing sugar data in full for the reason that no relation was observed between the treatment that was applied and the amount of reducing sugar obtained. A few of the results which are characteristic of the whole are shown in table 4. The values for the check lots show that the amounts of reducing sugar were quite different in different lots of potatoes, there being in some cases 15 times as much reducing sugar as in others; consequently, so far as the relation of composition to dormancy is concerned, the reducing sugar may be either high or low in two lots and both lots may be dormant. But the important question in these experiments is what effect the treatments have had upon the amount of reducing sugar that is present, irrespective of whether this is high or low at the beginning of the treatment. By comparing the values in table 4 it is seen that in no case was a series of values obtained corresponding to the series of concentrations of chemicals used in treating the potatoes. In this respect, therefore, the behavior of the reducing sugar was in contrast to that of the cane sugar. This is in agreement with measurements reported in the previous paper (2) in which there was no evidence of a consistent effect of the treatments in either increasing or decreasing the amount of reducing sugar.

TABLE 4. *Effect of Chemical Treatment of Potatoes Upon Reducing Sugar in Press-juice*

Ethylene Chlorhydrin Treatments		Sodium Thiocyanate Treatments		Thiourea Treatments	
Lot No.	Reducing Sugar in 5 cc. mg.	Lot No.	Reducing Sugar in 5 cc. mg.	Lot No.	Reducing Sugar in 5 cc. mg.
207	1.5	157	18.6	152	18.6
208	1.8	158	30.6	153	17.4
209	2.1	159	30.0	154	14.8
210	1.2*	160	23.1*	155	17.4*
255	1.4	235	17.7	224	15.1
254	1.5	234	18.3	225	7.2
252	2.3	233	18.4	226	13.8
253	1.5*	232	19.5*	227	3.9*

\* Check lot.

NOTE: For variety, source of tuber, time of digging corresponding to lot numbers, see table 1; and for concentrations of chemicals corresponding to lot numbers, see table 2.

### Starch Changes

The starch changes are shown in table 5. The lot numbers are the same as those for the sucrose data but the starch determinations were made upon dried and powdered whole tissue and not upon the press-juices. The percentages of starch are shown in columns 3, 4, 8, 9, 13, and 14 in table 5, columns 3, 8, and 13 showing the duplicate determinations and columns 4, 9, and 14 showing the averages of the two duplicates in each case. Columns 5, 10, and 15 in table 5 show the percentage change of treated with respect to the corresponding check lot in each series. Thus lot 137 was 4.8 percent lower than the check lot 141, this value being calculated, not as percent of starch in the tissue, but as percent of the check value in each experiment.

It will be observed that the highest concentration of chemical in each experimental series showed the lowest percentages of starch; this is true for all three chemicals. The starch in the treated when calculated with respect to the corresponding check showed losses varying in different experiments from 3.0 percent in lot 207 to 10.6 percent in lot 228. Lower concentrations of chemical showed smaller differences between treated and check, the differences in some cases being small and probably negligible, *e.g.*, lot Nos. 208, 209, 170, 238, 237, 180, and 230; in fact, two of the treated lots in which low concentrations of chemical were used, lot Nos. 139 and 140, showed higher starch values than the check lot No. 141.

Since the differences in starch values were small the values for the duplicate determinations are given in each case in order to take into account the question whether the differences between treated and check lots are in excess of the error of the determination itself. There are available 27 pairs of duplicate determinations, each pair of measurements having been made with a different lot of potato-powder. Fleisch (4) has proposed a method for estimating the analytical error in such cases, and, applying his method



should be emphasized. There is a tendency in the literature to class these two groups together as "sugar," and yet in these experiments there has been a qualitative difference between the response of the two, sucrose showing increases in a perfectly definite manner and the reducing sugar showing an irregular behavior.

It is not suggested that the breaking of dormancy is caused by the sucrose accumulation, and that growth starts when the sucrose content increases to a sufficient value. Soaking potatoes in a sucrose solution, or injecting it into the tissue even near the eye of the potato, does not induce growth of buds in dormant tubers. It seems more likely that the sucrose increase is mainly a result and not a cause. At present we can regard the sucrose increase only as evidence that the chemical treatments have become effective in the tissues of the tuber, and that subsequently sprouting of the buds will become evident.

These results which indicate an increase in sucrose following a decrease in starch, are of special interest in connection with the views of certain authors that there is some sort of a direct connection between starch and sucrose. This would not be expected in view of the fact that when starch is broken down by enzymes which can be separated from plant tissue maltose, not sucrose, is obtained. But there are several suggestions in the literature that a starch-sucrose equilibrium exists, and that sucrose is an intermediate product between starch and dextrose. The reader is referred to papers by Kayser (5), Ripperton (6), de Wolff (10), and Tollenaar (8) for further information on this point. It has often been found that as starch decreases sucrose increases; but it is merely an assumption to say that the connection is direct; it is better to say that we do not know what the steps are by which we may connect the disappearance of starch with the appearance of sucrose.

Although both the previous and present reports show that, in the treated lots as compared with the checks, starch decreased and sucrose increased, it should not be inferred that the changes in absolute amounts of these substances in the tissue were large. The actual changes were, in fact, relatively small. Thus, in the present experiments, assuming the moisture content of the tissue as 80 percent, and assuming that 80 cc. of press-juice are equivalent to 100 grams of fresh tissue, the excess loss of starch from a 25 gram treated seed-piece as compared with a check seed-piece was about 0.1 to 0.4 gram, and the gain in sucrose was about 0.05 to 0.2 gram. We may also recalculate the changes shown by the data from the previous report (2, table 2, p. 331) from which we find that the starch loss per 25 gram seed-piece was about 0.2 gram and the sucrose increase about 0.1 gram greater in the treated than in the check. These are relatively small absolute changes in material. Furthermore, these are the changes induced by favorable concentrations of chemical, and the data show that much lower concentrations have an observable effect not only upon the

sucrose and starch changes, but also upon the sprouting response. These facts show that the transition from dormancy to growth can take place without involving any extensive change in the total amounts of materials present in the tissue.

#### SUMMARY

1. Freshly harvested potatoes (*Solanum tuberosum* L.) were treated with ethylene chlorhydrin, sodium thiocyanate, and thiourea, the concentrations of the chemicals being decreased from the optimum by steps to form a graded series. Press-juices from the treated potatoes obtained from the various lots at a subsequent interval, usually four to seven days before sprouting became visible, were compared with juices from the checks with reference to sugar content.

2. Sucrose was found to be higher in the treated than in the check lots, and, furthermore, to form a graded series of values corresponding to the series of concentrations of chemicals used in treating the potatoes.

3. The reducing sugar values did not form such a series, and no consistent effect of the treatments in either increasing or decreasing the reducing sugar content was found.

4. When samples were taken at intervals of 24, 48, 72, etc., hours after treatment it was found that the time after treatment at which the sucrose content of the treated lots became higher than that of the checks differed in different experiments, being as early as 24 hours and as late as 72 hours.

5. Samples of entire tissue which had been dried, powdered, and analyzed for starch showed that concentrations of chemical favorable for breaking the dormancy of the sprouts caused decreases in the starch; with low concentrations of chemical, however, the differences were small and of doubtful significance.

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## THE TWIN-LEAF METHOD OF STUDYING CHANGES IN LEAVES<sup>1</sup>

F. E. DENNY

### INTRODUCTION

When measuring the changes which take place in leaves at intervals during a day by removing a sample of leaves at the beginning of the experimental period, and taking a second sample at the end of the period for comparison with the first one, it is important that the two samples be strictly comparable. If the differences between the two lots are to be taken as a measure of the change during the interval, we must be certain, first of all, that they were equal at the start.

So great is the variation in leaves upon a plant that to obtain two samples containing leaves of the same age, weight, and chemical composition by making a general collection requires a large number of leaves in order that these individual variations may be equalized. For many types of experiments this requirement can not be fulfilled because of the limitations set by space, by the numbers of plants available, by the details of technique involved in setting up the experiment, etc. If it is necessary to use relatively small numbers of plants, how shall we arrange to obtain the required number of comparable samples so that a series of samples over a considerable period of time may be obtained?

Sachs (9) offered a solution of this problem by proposing the use of what has now come to be known as the "half-leaf" method. He cut the leaf in two, lengthwise along the midrib, taking the first half as a sample to represent the condition at the beginning, and leaving the other half upon the plant for removal at the end of the desired period. Usually the entire half leaf was not used but a measured area was cut out using a template, and the results of the determination were expressed on the leaf area basis.

In later years this method was subjected to criticisms; first, that the opposite halves of the leaf were in fact not symmetrical, and further, that on account of fluctuations in area due to shrinkage when water was lost and to distention when water was gained, the computations on the leaf area basis were erroneous. Thoday (12) made these criticisms the object of a special investigation and found that both sources of error were important

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factors, that the asymmetry error was inherent in the method and could not be avoided, and that only the shrinkage error could be overcome. He suggested (13) an improvement which consisted in marking out at the beginning with a rubber stamp the area that was to be taken at the end of the period, thus nullifying the effect of any change in area that might occur during the experimental period.

Recently two more objections to the Sachs method have been made, these referring to errors arising from mutilation of the leaf in cutting away the half leaf or portion of it. Combes (4) claims that the wounding of the leaf in cutting increases the rapidity of translocation from the portion that remains, the effect being so great that he could distinguish between different degrees of wounding by the effect on translocation; and von Guttenberg (7) reports that removing a half leaf induces even qualitative changes in the composition of the remaining half; that, for example, in *Ilex* the wounding causes the appearance of saccharose in the other half leaf at a time when this sugar does not normally exist in it, and that a similar condition prevails as to glucosides in *Hedera*. He speaks of the method as leading to pathological conditions in the leaf.

There seems to be a need, therefore, for an alternative method that can avoid some of these difficulties of the half-leaf method. The present experiments were started for the purpose of testing the possibility of using the paired leaves of plants with opposite leaves or of leaflets of compound leaves, the plan in general being to take one leaf of the pair at the beginning of the experimental period, and the opposite one at the end. These opposite leaves are of exactly the same age, and, in some species at least, are so nearly the same in shape, size, and composition that we may regard them as twins. For this reason it is suggested that the method be called the "twin-leaf" method to distinguish it from the "half-leaf" method. And if it should be found from later more extensive tests that leaf pairs in general are not sufficiently alike to justify the word "twin" then the term "opposite-leaf" could be used.

Chibnall (3) used the opposite leaflets of *Phaseolus* in his study of the diurnal changes in nitrogen distribution, and Curtis (5) applied this method in measuring the effect of the cooling of petioles upon the rate of translocation from leaf blades. But both Chibnall and Curtis were interested in the use of the method for the purposes of their particular problems and not in the method itself; consequently they do not show in detail what the variation is between opposite leaves or leaflets, or make any effort to extend the application of the method to species other than the ones they used. It has been the purpose of the present experiments to explore the field in this direction, to measure the amount of variation likely to be encountered in paired leaves, to note by means of chemical analysis to what extent opposite leaves have the same composition, and finally to apply the method to the problem of determining the changes in leaves at intervals during a 24 hour period.

## AMOUNT OF VARIATION IN OPPOSITE LEAVES

In order to determine the variation in weight of opposite leaves of various species, samples of paired leaves of the species shown in table 1 were taken. In obtaining the samples the two leaves of each pair were

TABLE 1. *Variations in Fresh Weights of Opposite Leaves or Leaflets*

Species	Fresh Weights of Opposite Leaves,† Grams		% Total Dev.
	a	b	
<i>Coleus Blumei</i> Benth. (yellow).....	13.913	14.341	3.1
<i>Coleus Blumei</i> Benth. (varieg.).....	7.424	7.374	0.7
<i>Glycine Max</i> Merr.....	8.078	8.023	0.7
<i>Glycine Max</i> Merr.....	8.013	8.052	0.5
<i>Rosa rugosa</i> Thunb.....	5.132	5.117	0.3
<i>Rosa rugosa</i> Thunb.....	4.944	4.923	0.4
<i>Ailanthus altissima</i> Swingle.....	5.496	5.554	1.1
<i>Ailanthus altissima</i> Swingle.....	7.678	8.013	4.2
<i>Gardenia jasminoides</i> Ellis.....	11.660	11.209	3.9
<i>Bryophyllum calycinum</i> Sal.sb.....	39.857	40.482	1.8
<i>Syringa vulgaris</i> L.....	5.120	5.274	3.0
<i>Helianthus debilis</i> Nutt.*.....	29.504	29.355	0.5
<i>Lonicera Standishii</i> Carr.....	4.059	4.213	3.8
<i>Melilotus alba</i> Desr.....	1.422	1.453	2.2
<i>Melilotus alba</i> Desr.....	1.245	1.225	1.6
<i>Deutzia gracilis</i> Sieb. and Zucc.....	5.524	5.305	4.0
<i>Ligustrum ovalifolium</i> Hassk.....	4.784	4.738	1.0
<i>Ligustrum ovalifolium</i> Hassk.....	4.043	4.088	1.1

\* Cultivated form of sunflower, listed in the seed catalog as belonging to the cucumerifolius type. This form has opposite leaves only in the early stages of growth.

† In all cases both samples collected at the same time.

NOTE: 25 pairs of leaves or leaflets in each case except for *Melilotus* in which case 50 pairs of leaflets were used.

picked simultaneously, and were put in separate weighing bottles; this was repeated until 25 pairs of leaves were collected; these were then weighed and in table 1, the second column (under *a*) shows the total fresh weight of 25 leaves or leaflets, and the third column (under *b*) shows the total weight of the opposite organs. In column 4 will be found the percentage total deviation between the two samples, *i.e.*, the percent error that results from the assumption that the opposite leaves of a composite sample of 25 pairs of leaves are identical in weight. This value ranges in the experiment from 0.3 percent with *Rosa rugosa* to 4.2 percent with *Ailanthus jasminoides*. We can obtain an estimate of the amount of the error that would likely result if composite samples of 16, 9, or 4 leaves instead of 25 were taken by making use of the general relation that the error is inversely proportional to the square root of the number. The error for 16 leaves would then be about five-fourths, of nine leaves about five-thirds, etc., of the values shown in column 4 in table 1.

TABLE 2. Variation in Weights of Opposite Leaves of Pairs

Leaves of Pair	Salvia splendens Ker.														Ligustrum ovalifolium Hassk.		Helianthus debilis Nutt.	
	Tip Leaves							Pair of Leaves Below Tip							Tnird Pair		Fresh Wt. g.	% dev.*
	Fresh Wt. g.	% dev.*	Dry Wt. g.	% dev.*	Fresh Wt. g.	% dev.*	Dry Wt. g.	% dev.*	Fresh Wt. g.	% dev.*	Dry Wt. g.	% dev.*	Fresh Wt. g.	% dev.*	Fresh Wt. g.	% dev.*	Fresh Wt. g.	% dev.*
a	0.126	4.8	0.019	0.0	0.353	8.0	0.044	4.5	0.657	1.5	0.094	1.0	0.196	2.0	0.943	1.6		
b	0.132		0.019		0.325		0.042		0.647		0.095		0.192		0.928			
a	0.144	9.1	0.021	9.5	0.361	4.1	0.051	3.9	0.557	10.0	0.080	8.8	0.253	1.6	1.356	3.8		
b	0.157		0.023		0.348		0.049		0.501		0.073		0.257		1.408			
a	0.197	4.6	0.023	13.0	0.318	7.2	0.045	2.2	0.551	4.3	0.066	9.1	0.179	3.3	0.784	2.1		
b	0.206		0.026		0.341		0.046		0.575		0.072		0.185		0.768			
a	0.165	1.2	0.026	7.7	0.338	2.4	0.047	4.2	0.520	3.7	0.077	5.2	0.231	6.9	0.803	9.3		
b	0.163		0.024		0.330		0.045		0.539		0.081		0.215		0.728			
a	0.137	2.2	0.021	0.0	0.445	0.2	0.063	3.1	0.593	0.3	0.069	4.3	0.179	0.0	0.919	1.5		
b	0.134		0.021		0.441		0.065		0.591		0.072		0.179		0.933			
a	0.162	4.3	0.026	3.8	0.306	1.0	0.047	8.5	0.848	4.4	0.117	4.3	0.204	1.0	0.898	1.4		
b	0.169		0.027		0.303		0.043		0.811		0.112		0.206		0.910			
a	0.146	2.0	0.025	0.0	0.278	7.5	0.038	7.9	0.647	4.6	0.104	1.0	0.232	9.9	1.074	6.9		
b	0.143		0.025		0.299		0.041		0.677		0.103		0.255		1.148			
a	0.230	3.9	0.029	3.4	0.408	2.5	0.055	1.8	0.724	1.7	0.093	3.2	0.190	13.6	0.924	7.0		
b	0.221		0.028		0.418		0.054		0.712		0.090		0.174		0.860			
a	0.176	1.1	0.027	0.0	0.415	2.9	0.059	1.7	0.548	4.0	0.071	1.4	0.157	12.8	1.071	9.6		
b	0.178		0.027		0.427		0.060		0.526		0.070		0.177		0.968			
a	0.147	8.1	0.019	5.3	0.353	8.5	0.047	8.5	0.530	3.9	0.064	7.8	0.275	8.0	0.830	1.7		
b	0.159		0.020		0.383		0.051		0.551		0.069		0.253		0.844			
Ave. % dev. 4.1				4.3		4.4		4.6		3.8		4.6		5.9				

\* Percent deviation is difference between *a* and *b* expressed as percent of *a*.NOTES: In each case *a* refers to one leaf and *b* to the opposite leaf of this pair, the leaves being taken simultaneously.

The comparison was carried out in greater detail with leaves of *Salvia*, *Ligustrum*, and *Helianthus* as shown in table 2, in which will be found the weights of individual leaves. Thus, the two leaves at the tip of *Salvia* were weighed separately as shown in column 2 opposite *a* and *b*, respectively; then follow in column 4 the dry weights. The data for ten pairs of leaves from the tips of *Salvia* plants are thus shown in columns 2, 3, 4, and 5; and in columns 6, 7, 8, and 9 are shown the results of similar measurements for the paired leaves below the tip, etc. Also in the right hand columns of table 2 are shown the measurements with *Ligustrum* and *Helianthus*.

The percentage deviations of one leaf from the opposite leaf are shown in columns 3, 5, 7, 9, 11, 13, 15, and 17, table 2. These values are similar for the different types of leaves and are about four to six percent. This is the variation per single pair of leaves. If the sample was taken by combining 9, 16, or 25 pairs of such leaves the error would be reduced to approximately one-third, one-fourth, and one-fifth these values, respectively.

We may compare these values (which are errors due to lack of symmetry between opposite leaves) with the values obtained for asymmetry between opposite halves of the same leaf. Thoday (12) made measurements of the deviation of the weight of one half leaf from that of the other half, and found results which varied with different species but was commonly about 1.5 to 3.0 percent and sometimes more than 4.0 percent. Gouwentak (6) in a recent report on a study of diurnal changes in the nitrogen contents of *Helianthus* leaves gives (6, p. 44) the dry weights of opposite halves of leaves. There were eight leaves in the comparison, and while Gouwentak expresses the data on the grams-per-square-decimeter basis, we may calculate the values in the manner that was done for the opposite leaves of *Salvia*, i.e., by expressing the total deviation between the two halves as a percentage of the value for the leaf half marked *a* in Gouwentak's list. This gives the average percentage deviation between leaf halves as 3.2 percent. The values found by Thoday and by Gouwentak for the half-leaf method are, therefore, somewhat lower than the asymmetry errors of the twin-leaf method, as shown by tables 1 and 2 above, but the difference is not large, and considering the small numbers involved it can not be asserted that even this difference is significant. The twin-leaf method gives sufficiently low error values to warrant its consideration as a method of getting comparable samples of leaves.

#### · VARIATION IN CHEMICAL COMPOSITION OF SAMPLES OF OPPOSITE LEAVES

Table 3 shows the differences in chemical composition of samples of opposite leaves, the differences in these cases representing not only errors in the sampling but also errors in the analyses. The *A* series was carried out in 1929 with composite samples of 30 leaves and the *B* series in 1930

TABLE 3. Chemical Composition of Samples of Opposite Leaves of *Sabina*. Series A in April 1929

	Experiment No. 1				Experiment No. 2				Experiment No. 3				Experiment No. 4				Experiment No. 5			
	Total in 30 Leaves (grams)		% of the Fresh Wt.		Total in 30 Leaves (grams)		% of the Fresh Wt.		Total in 30 Leaves (grams)		% of the Fresh Wt.		Total in 30 Leaves (grams)		% of the Fresh Wt.		Total in 30 Leaves (grams)		% of the Fresh Wt.	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Fresh Wt. ....	18.432	18.266			12.006	12.058			12.428	12.209			10.249	10.583			11.779	11.749		
Dry Wt. ....	2.433	2.413	13.2	13.2	1.651	1.639	13.8	13.6	1.706	1.695	13.7	13.8	1.540	1.611	15.0	15.2	1.602	1.600	13.6	13.6
Water ....	15.999	15.853	86.8	86.8	10.355	10.419	86.2	86.4	10.722	10.604	86.3	86.2	8.709	8.972	85.0	84.8	10.177	10.149	86.4	86.4
Insol. Solids	1.604	1.602	8.6	8.7	1.380	1.367	11.5	11.3	1.446	1.428	11.7	11.6	1.257	1.338	12.3	12.8	1.312	1.321	11.2	11.2
Sol. Solids...	0.829	0.811	4.5	4.4	0.271	0.272	2.6	2.3	0.260	0.267	2.2	2.2	0.283	0.273	2.8	2.6	0.290	0.279	2.5	2.5
Starch ....	0.560	0.541	3.04	2.98	0.555	0.564	4.62	4.65	0.644	0.652	5.18	5.30	0.617	0.633	6.06	6.03	0.610	0.603	5.22	5.22
Insol. N. ....	0.108	0.112	0.59	0.62	0.061	0.058	0.51	0.48	0.052	0.054	0.42	0.44	0.043	0.046	0.42	0.44	0.045	0.048	0.39	0.39

## Series B in May 1930

	Experiment 6 Tip Leaves				Experiment 7 Leaves Below Tip				Experiment 8 Third Pair of Leaves				Experiment 9 Tip Leaves				Experiment 10 Leaves Below Tip				Experiment 11 Third Pair of Leaves			
	Total in 15 Leaves (grams)		% of the Fresh Wt.		Total in 10 Leaves (grams)		% of the Fresh Wt.		Total in 10 Leaves (grams)		% of the Fresh Wt.		Total in 15 Leaves (grams)		% of the Fresh Wt.		Total in 10 Leaves (grams)		% of the Fresh Wt.		Total in 10 Leaves (grams)		% of the Fresh Wt.	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Fresh Wt. ....	2.631	2.614			3.710	3.639			6.041	6.129			1.630	1.662			3.575	3.618			6.175	6.130		
Dry Wt. ....	0.384	0.383	14.7	14.7	0.522	0.507	14.1	13.9	0.822	0.839	13.5	13.7	0.236	0.240	14.5	14.4	0.452	0.454	17.5	17.3	0.835	0.837	13.5	13.5
Starch ....	0.025	0.027	0.95	1.03	0.034	0.035	0.92	0.96	0.052	0.051	0.86	0.83	0.023	0.023	1.41	1.38	0.047	0.042	1.31	1.16	0.073	0.076	1.18	1.18
Red. Sugar	6.0*	6.3*	0.23	0.24	6.2*	6.3*	0.17	0.18	8.7*	9.5*	0.14	0.16	2.9*	3.0*	0.18	0.18	5.9*	5.9*	0.16	0.16	7.7*	7.2*	0.12	0.12
Sucrose ....	1.8*	1.3*	0.07	0.05	5.8*	4.8*	0.16	0.13	9.9*	5.3*	0.16	0.09	2.1*	3.4*	0.13	0.20	2.1*	2.9*	0.06	0.08	4.1*	5.4*	0.07	0.07
Insol. N. ....	0.015	0.016	0.58	0.61	0.021	0.021	0.57	0.57	0.031	0.029	0.52	0.47	0.010	0.010	0.61	0.60	0.020	0.019	0.54	0.52	0.031	0.030	0.50	0.50

\* Milligrams instead of grams.

NOTE: Leaves in experiments 9, 10, and 11 dried in an electric oven at 99° C.



with composites of 10 to 15 leaves. It is seen that good agreement was obtained with constituents which represented fairly large weights of material per sample such as fresh weight, dry weight, and starch, but that with sugars the percentage error was large, due to the small amounts of these substances in the tissue.

#### METHODS OF ANALYSIS

For the experiments in April, 1929, the leaves (with petioles removed), after being weighed, were cut into pieces and dropped into boiling alcohol of sufficient volume to give a final concentration of 80 percent alcohol. When the process of extraction was started the liquid was decanted through a tared Soxhlet extraction thimble and finally all particles of tissue were transferred to it; the tissue was then extracted with alcohol in the Soxhlet apparatus and the extracts made up to volume. The residue in the thimble was dried in an oven at 99° C.; the difference in weight in comparison with the tared thimble gave the weight of insoluble solids; an aliquot of the extract was taken for the determination of the soluble solids; the sum of the insoluble solids and the soluble solids gave the dry weight, and the dry weight and fresh weight difference gave the water content. Samples from the residue which was insoluble under the conditions of the extraction were taken for insoluble nitrogen by the Kjeldahl method (1, p. 7), and also for the starch determination. This was carried out by the acid-hydrolysis method (1, p. 95) except in one case in which the Walton and Coe method (14) was used.

In the May, 1930, experiments the samples after being dropped into boiling alcohol were put into a weighed porcelain dish on a steam bath until the alcohol was evaporated; they were then dried in a vacuum oven at 70° C. The difference in weight gave dry weight for the leaves representing the second pair below the tip, but this value could not be obtained for the tip leaves and for the leaves representing the first pair below the tip in this experiment, since calcium carbonate had been added to the alcohol at the time the leaves were dropped into it. The dried residue was then transferred first to a mortar and ground up with 70 percent alcohol, and then to pyrex centrifuge tubes. After the liquid had been brought to boiling in the 70 percent alcohol and then allowed to cool, it was centrifuged and the extract was decanted; another quantity of 70 percent alcohol was added and a second extraction was made; in this way six extractions were carried out. The extracts were combined and aliquots were used for the sugar and for soluble nitrogen determinations. The entire residue was used for both starch (or more accurately acid-hydrolyzable polysaccharids) and insoluble nitrogen in the following manner: After the period of acid-hydrolysis the liquid was removed by successively centrifuging and decanting; the liquids were collected, neutralized, made up to volume, and five cc. samples taken for the Somogyi (11) modification of the Shaffer and Hartman micromethod (10); the residue and the liquid portion were digested

separately in Kjeldahl flasks; the digest-liquid from the residue was made up to volume and an aliquot of this exactly equal to the aliquot of the liquid portion was taken; these two digests were then recombined and represented a given aliquot of the original material; a Kjeldahl distillation then gave the amount of nitrogen in the insoluble portion of the sample of leaves.

## DIURNAL CHANGES IN LEAVES

### General Procedure

*Salvia splendens* Ker. was chosen for this experiment in which the object was to make use of the twin-leaf method for studying the changes in leaves at intervals of 2.5 to 4.0 hours throughout a 24 hour period. The plants were grown from seed, were about six to eight inches high, and had produced several pairs of leaves. When the plants are young the opposite leaves of *Salvia* are very uniform, but when they get older and when branching begins the leaves become coarser and less symmetrical.

Three types of leaves were sampled, the young tip leaves, the leaves of the pair below the tip, and the third pair of leaves; these represent the very young, the partly grown, and nearly full grown stages of leaves. The samples of the three types were kept separate, and one of the interesting results of the experiment has been to note the difference in the behavior of the three types.

The first experiment for diurnal changes was begun on April 3, 1929, samples being taken at 5:30, 8:00, 10:30 A.M., 1:00, 3:30, 7:00, 11:00 P.M. and 4:00 A.M. April 3 was a bright and sunny day. Thirty leaves were taken for each sample and the procedure in getting a series of comparable leaves throughout the day was as follows: at 5:30 A.M. one leaf of each of 30 pairs of opposite leaves was taken and the other 30 leaves were left until 8:00 A.M., at which time this second sample was collected; but at the same time another sample of 30 leaves from 30 other plants was taken, these representing the first sample for the period 8:00 A.M. to 10:30 A.M., the opposite leaves being left on for the sample at the end of the period. In this way samples at the beginning and end of each period were available from twin-leaves, and the change during each period could be deduced from the differences in these pairs, and expressed in absolute amounts of material, *i.e.*, grams of water, dry weight, starch, sugar, etc., or as the percentage of the fresh weight if this was considered advisable, since the data for this computation were available. In collecting each sample of leaves an effort was made to obtain leaves with varying positions toward the sun in order to equalize variations in this respect.

Another test was made May 12, 1930, sampling starting at 6:00 A.M. and continuing until 5:00 A.M., May 13. The method of sampling was the same as described for April 1929 except that a smaller number of leaves per sample was used, 20 of the tip-leaves and of the pair below the tip,

and 15 of the third pair of leaves. May 12 was entirely clear throughout the day.

### Results of the April 1929 Experiment

The data for the April 1929 test are given in tables 4, 5, and 6 which show the total number of grams of material in each sample, the percentage change of each constituent, and the total amount at any period calculated with reference to the amount present at the start in the early morning. Table 4 shows the data for the tip-leaves, table 5 for the leaves just below the tip, and table 6 for the third pair of leaves (second below the tip).

TABLE 4. *Diurnal Changes in Composition of Salvia Leaves. Tip Leaves. April 1929 Experiment*

Time	Total Amount in 30 Leaves			Percent Gain (+) or Loss (-) During the Period *			Relative Amount Present. Amount at 5:30 A.M. as 100		
	Fresh Wt. grams	Dry Wt. grams	Water grams	Fresh Wt.	Dry Wt.	Water	Fresh Wt.	Dry Wt.	Water
{ 5:30 A.M.	7.164	0.898	6.266						
{ 8:00 A.M.	7.346	0.952	6.394	+ 2.5	+ 6.0	+ 2.0	103	106	102
{ 8:00 A.M.	6.963	0.947	6.016						
{ 10:30 A.M.	7.161	1.055	6.106	+ 2.8	+ 11.4	+ 1.5	105	118	104
{ 10:30 A.M.	6.702	1.062	5.640						
{ 1:00 P.M.	7.179	1.159	6.020	+ 7.1	+ 9.1	+ 6.8	113	129	111
{ 1:00 P.M.	6.425	1.075	5.350						
{ 3:30 P.M.	7.130	1.185	5.945	+ 11.0	+ 10.2	+ 11.3	125	142	123
{ 3:30 P.M.	7.893	1.336	6.557						
{ 7:00 P.M.	8.134	1.308	6.826	+ 3.1	- 2.1	+ 4.1	129	139	128
{ 7:00 P.M.	8.593	1.387	7.206						
{ 11:00 P.M.	8.996	1.374	7.622	+ 4.7	- 1.0	+ 5.8	134	138	130
{ 11:00 P.M.	9.718	1.460	8.258						
{ 4:00 A.M.	9.718	1.401	8.317	0	- 4.0	+ 0.7	134	132	129

\* Percent change in each interval calculated on the amount present at the beginning of that period.

NOTE: Brackets indicate twin-leaves.

The columns in tables 4, 5, and 6 which show the total weights in 30 leaves are self-explanatory. In the central columns in each table the percentage gain or loss in the amount of the constituent is based upon the amount present at the beginning of that period; thus, in table 5, column 9, the dry weight changed from 2.125 g. at 5:30 A.M. to 2.198 g. at 8:00 A.M.; the difference is 0.073 g. which is 3.4 percent of 2.125. In this way the changes during each period may be plotted as has been done, for example, in text figure 3; such a graph is a rate-curve and shows when the rate of change is the highest, and when it reverses in sign, if at all, etc.

TABLE 5. *Diurnal Changes in the Composition of Sabia Leaves. Leaves Below Tip. April 1929 Experiment*

Time	Total Amount in 30 Leaves						Percent Gain (+) or Loss (-) During the Interval *				Relative Amount at End of Interval, Amount at 5:30 A.M. as 100				
	Fresh Wt. g.	Dry Wt. g.	Water g.	Starch g.	Sol. Solids g.	Insol. N. mg.	Fresh Wt.	Dry Wt.	Water	Starch	Fresh Wt.	Dry Wt.	Water	Starch	Sol. Solids
{ 5:30 A.M. 8:00 A.M.	17.467	2.125	15.342	0.339	0.800	102	-0.5	+ 3.4	-1.1	+13.0	99	103	99	113	102
	17.377	2.198	15.179	0.383	0.817	99									
{ 8:00 A.M. 10:30 A.M.	15.349	1.961	13.388	0.339	0.709	95	-0.3	+10.0	-1.8	+45.0	99	113	97	164	113
	15.305	2.157	13.148	0.491	0.790	93									
{ 10:30 A.M. 1:00 P.M.	15.570	2.281	13.289	0.514	0.813	98	+5.6	+15.3	+3.9	+39.2	105	130	102	228	122
	16.437	2.630	13.807	0.715	0.877	116									
{ 1:00 P.M. 3:30 P.M.	15.893	2.491	13.402	0.756	0.883	101	+4.2	+ 5.6	+3.9	+ 1.4	109	138	106	231	125
	16.552	2.630	13.922	0.766	0.907	106									
{ 3:30 P.M. 7:00 P.M.	17.441	2.802	14.639	0.902	0.924	108	+4.5	- 1.9	+5.7	- 7.5	113	135	112	214	110
	18.217	2.749	15.468	0.835	0.812	115									
{ 7:00 P.M. 11:00 P.M.	17.461	2.738	14.723	0.856	0.876	112	+2.5	- 4.6	+3.9	-24.0	116	129	116	163	107
	17.902	2.612	15.290	0.647	0.856	107									
{ 11:00 P.M. 4:00 A.M.	19.202	2.715	16.487	0.811	0.868	112	-1.0	- 5.7	-0.3	-22.0	115	122	116	127	102
	19.004	2.559	16.445	0.631	0.821	111									

\* Percent change in each interval calculated on the amount present at the beginning of that period.

NOTE: Brackets indicate twin-leaves.

TABLE 6. *Diurnal Changes in the Composition of Sabia Leaves. Third Pair (Second Pair of Leaves Below Tip). April 1920 Experiment*

Time	Total Amount in 30 Leaves							Percent Gain (+) or Loss (-) During the Interval*				Relative Amount at End of Interval, Amount at 5:30 A.M. as 100			
	Fresh Wt. g.	Dry Wt. g.	Water g.	Starch g.†	Sol. Solids g.	Sol. N. mg.	Insol. N. mg.	Fresh Wt.	Dry Wt.	Water	Starch	Fresh Wt.	Dry Wt.	Starch	Sol. Solids
{ 5:30 A.M. ....	22.307	2.609	19.698	0.149	0.962	14.4	121	-1.2	+ 3.2	-1.5	+ 4.7	99	103	105	104
{ 8:00 A.M. ....	22.101	2.694	19.407	0.156	1.001	14.7	123								
{ 8:00 A.M. ....	19.792	2.395	17.397	0.152	0.817	12.5	113	-3.9	+15.9	-6.7	+91.0	95	120	201	112
{ 10:30 A.M. ....	19.017	2.792	16.225	0.290	0.883	13.6	117								
{ 10:30 A.M. ....	19.564	2.778	16.786	0.290	0.963	12.8	120	+4.5	+ 9.1	+3.6	+49.7	99	131	300	114
{ 1:00 P.M. ....	20.425	3.064	17.361	0.473	1.016	14.8	125	+4.1	+ 5.1	+4.0	+14.6	103	137	344	117
{ 3:30 P.M. ....	21.272	3.220	18.052	0.542	1.046	15.7	119								
{ 3:30 P.M. ....	22.220	3.409	18.811	0.567	1.071	16.8	133	+3.6	+ 0.2	+4.2	- 6.5	107	138	322	112
{ 7:00 P.M. ....	23.021	3.416	19.605	0.530	1.024	16.6	136								
{ 7:00 P.M. ....	21.128	3.126	18.002	0.491	0.966	15.9	123	+1.9	- 4.1	+8.5	-19.4	109	132	259	113
{ 11:00 P.M. ....	21.537	2.999	19.538	0.394	0.971	15.9	122								
{ 11:00 P.M. ....	21.647	3.065	18.582	0.397	1.009	15.5	123	-2.4	- 7.9	-1.5	-24.9	106	121	195	103
{ 4:00 A.M. ....	21.120	2.821	18.299	0.299	0.910	14.2	125								

\* Percent change in each interval calculated on the amount present at the beginning of that period.

† By Walton and Coe (14) method.

NOTE: Brackets indicate twin-leaves.

The right hand columns in tables 4, 5, and 6 show the percentage at the end of any given period when the amount at the start of the experiment is placed at 100; thus, in table 4, column 9, the dry weight at 5:30 A.M. is 100, and since the gain from 5:30 to 8:00 was 6.0 percent (see column 6), the amount at 8:00 is 106; and since the gain from 8:00 to 10:30 was 11.4 percent of the amount at 8:00 the percentage at 10:30 with reference to the start was  $106 + (0.114 \times 106) = 118$ . In this way the values showing the relations between the amount at any time and the amount at the start of the day were obtained (see text figs. 1 and 2).

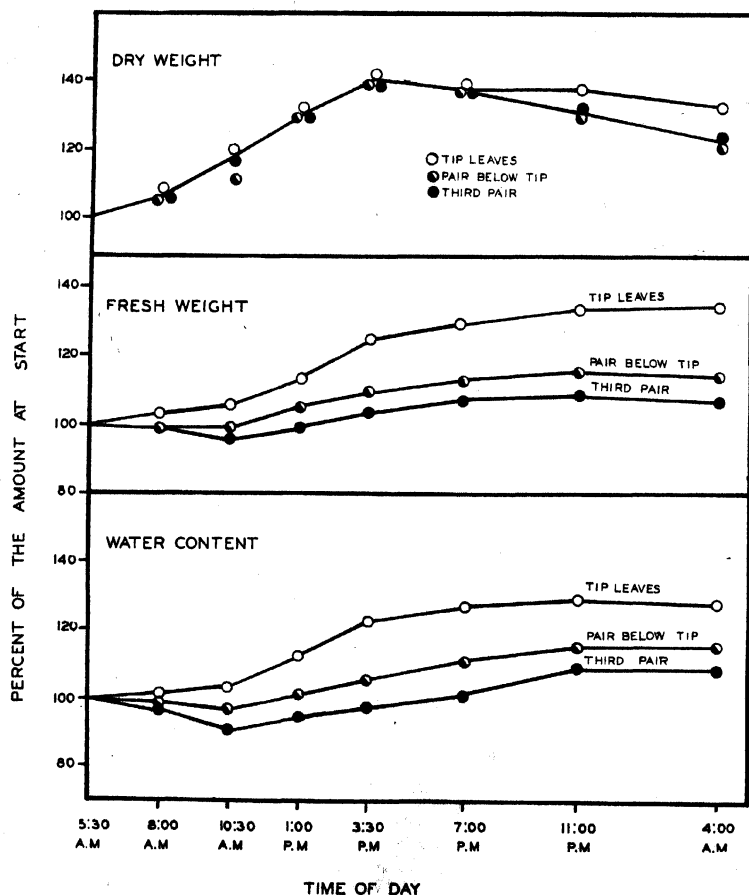
There is a danger in this method of procedure, in that it tends to pyramid values, and to propagate throughout the daily record any erroneous reading that may have been taken. But the occurrence of any large error can be detected by calculating the fresh weight percentage for the constituent at each sampling period. Thus, with the exception of the first and last samples, there are available always two simultaneous samples at each period, and, although these two were obtained by taking leaves from different plants, the fresh weight percentages should be reasonably nearly the same; in this way the occurrence of any large error can be detected, and its propagation throughout the day can be avoided. Although these calculated values do not have the dependability of the original data they show the general change throughout the day, and permit the construction of curves such as text figures 1 and 2, which have been built up into a continuous curve from the step-wise measurements of the individual periods.

Another method of collecting samples so as to avoid this propagation of errors would be to pick at the beginning one leaf from each of all the pairs to be used during the experiment, keeping them in as many groups as there are subsequent sampling periods, and then to pick the corresponding pairs at intervals thereafter. The differences for different periods will then be the total difference over the entire period, and there will be no propagation of error. This has the disadvantage that no samples except the first will be simultaneous, and consequently no opportunity is had to check against accidental errors by computing the fresh weight percentages of the simultaneous samples of non-twin leaves.

#### *Changes in Fresh Weight, Dry Weight, and Water Content*

Tables 4, 5, 6 and text figure 1 show an interesting difference in the behavior of the three types of leaves with reference to fresh weight, dry weight, and water content. The tip leaves did not decrease in fresh weight or water content at any time during the day but gained continually; the pair below the tip and the third pair lost water early in the morning up to about 10:30, at which time they began to gain in water, but the pair below the tip did not recover the water previously lost until about 1:00 P.M., and the third pair not until about 5:00 P.M. The fresh weight change reflected the moisture change, being lowered somewhat in the early forenoon

and rising again during the night. Although the three types of leaves differed with respect to fresh weight and moisture changes, the dry weight changes were very similar (see table 4, column 6; table 5, column 9; table 6, column 10, and text fig. 1). Even though the leaf weights of the types were quite different, the percentage increases during the first few periods

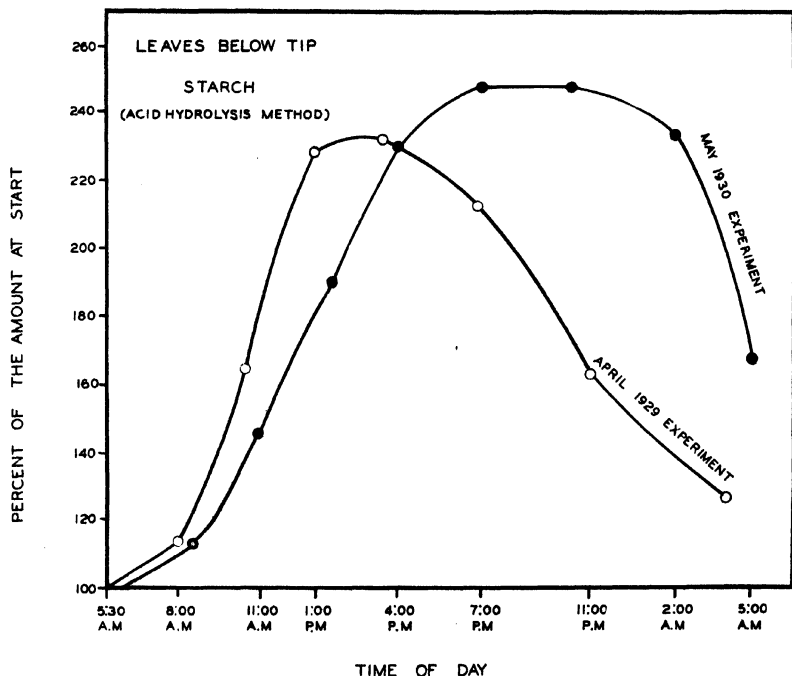


TEXT FIG. 1. Amount present at the end of each interval with reference to the amount present at the start. Notice differences in the behavior of tip leaves, leaves below the tip, and the third pair of leaves, i.e., second pair below the tip. *Salvia splendens* Ker.

were similar numerically; during the night, however, the fall in dry weight was not so fast in the tip leaves as in the other types (see text fig. 1). All three types had higher dry weights in the early morning of the second day than they had at the start of the experiment, whether the calculation is made on the twin leaf basis or on the percentage of the fresh weight basis.

### Starch

Although the term starch is applied throughout this paper, the method mainly used was that of acid-hydrolysis (see 1, p. 95), and consequently the determination includes not only true starch but also all other compounds that hydrolyze with dilute acid to give copper reducing substances. In the present experiments the purpose was to test the use of the twin-leaf method for studying the changes of substances, and emphasis was not put on the nature of the substances themselves; consequently this grouping of substances will not seriously disturb the general conclusions. The Walton



TEXT FIG. 2. Starch by acid-hydrolysis method in leaves below the tip with reference to the amount present at the start. Notice that time of attainment of the maximum and of the beginning of rapid translocation was early in the 1929 experiment and late in the 1930 experiment. *Salvia splendens* Ker.

and Coe (14) method, which eliminates interfering polysaccharids, was used in one test (third pair of leaves in the 1929 experiment), but there was doubt as to whether the enzyme was bringing about complete hydrolysis of the starch. Subsequent determinations showed about 25 percent lower values by the Walton and Coe procedure than by the acid-hydrolysis method. No doubt a study of changes in the non-starch acid-hydrolyzable polysaccharids would give interesting results, and it appears likely that the twin-leaf method would be well suited for this purpose.



The quantity of starch (or the acid-hydrolyzable substances) found in each sample is shown in table 5, column 5, and table 6, column 5. The percentage increases during each experimental period are shown in column 11, table 5, and in column 12, table 6. The greatest percentage increases occurred in the period from about 8:00 to 10:30 A.M., but the maximum amount of starch in the tissue was not reached until later in the day, about 1:00 to 3:30 P.M.

In text figure 2 will be found the relative amounts of starch present in the leaves at the various intervals during the day with reference to the amount present in the early morning.

The data in tables 5, 6, and 7*B* show increases during a 2.5 hour period of 30 to 90 percent of the amount of acid-hydrolyzable substances present at the beginning of the period; this indicates that it would be possible to measure this increase over a much shorter period, possibly during a 30 minute interval.

#### *Soluble Solids*

The weights of material soluble under the conditions of extraction used in these experiments are shown in table 5, column 6, and table 6, column 6; they are of interest in showing that the soluble solids made a complete excursion during the 24 hour period, increasing in amount up to about 2:00 P.M., and then decreasing so that the amount present the next morning was about the same as that at the beginning of the experiment.

#### *Insoluble Nitrogen*

The changes in the amounts of nitrogen in the insoluble fraction were so small that it can not be definitely stated whether any change at all occurred. As shown in table 5, column 7, and in table 6, column 8, the absolute amounts in 30 leaves underwent small changes during each experimental period but it seems unlikely that these differences are significant. Furthermore, when these amounts are calculated on the percentage of fresh weight basis, it is found that the range of values including both types of leaves and at all sampling periods throughout the day was only from 0.54 percent to 0.71 percent of the fresh weight. There was a tendency for slightly higher values at about noon and lower values in the early morning. But it would require larger samples with the resulting smaller experimental errors to obtain dependable values for showing the change in insoluble nitrogen.

#### **Results of the May 1930 Experiment**

The results of the May 1930 experiment are shown in tables 7*A* and 7*B*. Table 7*A* gives the total amounts of substances found in the entire sample at each experimental period, and table 7*B* shows the percentage change during each period, and the relation of the value at any time to that at the beginning of the experiment.

TABLE 7 A. *Changes in Salvia Leaves During a Day and Night. May 1930 Experiment*

Time	Tip Leaves	Leaves Below Tip			Third Pair (Second Pair Below Tip)					
	Total in 20 Leaves	Total in 20 Leaves			Total in 15 Leaves					
	Fresh Wt. g.	Fresh Wt. g.	Starch g.	Insol. N. mg.	Fresh Wt. g.	Dry Wt. g.	Water g.	Starch g.	Sol. N. mg.	Insol. N. mg.
{ 6:00 A.M..... 8:30 A.M.....	4.235 3.985	9.575 8.925	0.148 0.166	52 54	8.494 7.631	1.048 1.083	7.446 6.548	0.170 0.188	4.6 4.2	39 39
{ 8:30 A.M..... 11:00 A.M.....	4.085 4.135	9.860 9.948	0.160 0.207	60 61	9.253 9.177	1.261 1.353	7.992 7.824	0.196 0.283	4.3 5.0	47 49
{ 11:00 A.M..... 1:30 P.M.....	3.716 3.859	8.945 9.324	0.178 0.232	57 60	7.978 8.064	1.164 1.282	6.814 6.782	0.200 0.260	5.8 5.2	43 45
{ 1:30 P.M..... 4:00 P.M.....	3.632 3.865	9.315 9.723	0.218 0.264	58 lost	8.471 8.742	1.366 1.491	7.105 7.251	0.289 0.307	5.0 lost	46 48
{ 4:00 P.M..... 7:00 P.M.....	3.213 3.340	8.562 8.862	0.238 0.257	56 56	8.347 8.619	1.409 1.479	6.938 7.140	0.296 0.296	4.6 4.9	44 45
{ 7:00 P.M..... 10:30 P.M.....	3.515 3.659	7.902 8.110	0.227 0.227	49 49	7.814 7.676	1.265 1.234	6.549 6.442	0.273 0.273	4.2 4.0	41 40
{ 10:30 P.M..... 2:00 A.M.....	3.669 3.654	8.562 8.453	0.196 0.180	lost 53	7.896 7.649	1.255 1.131	6.641 6.518	0.282 0.208	4.5 4.1	41 41
{ 2:00 A.M..... 5:00 A.M.....	3.784 3.952	9.137 9.247	0.212 0.151	56 52	8.637 8.697	1.333 1.241	7.304 7.456	0.242 0.197	3.4 4.4	46 44

NOTE: Brackets indicate twin-leaves.

*Fresh Weight*

Columns 2, 3, and 6 in table 7A, and columns 2, 3, and 5 in table 7B show that the fresh weight behavior was similar in most respects to that noted in the 1929 experiment. The fresh weight losses in the early morning hours were greater in the 1930 experiment than in that of 1929, and the gain during the night was not as great. The percentage changes during the different periods are shown for the third pair of leaves in text figure 3.

*Dry Weight*

The dry weight values for the third pair of leaves are shown in table 7A, column 7, and in table 7B, columns 6 and 13. They are of special interest in comparison with the results from the 1929 experiment in showing maximum dry weight values much later in the day. Thus, in 1929 this maximum was reached by mid-afternoon but in 1930 it did not occur until about 7:00 P.M. The percentage changes in dry weight during different intervals are shown for the third pair of leaves in text figure 3.

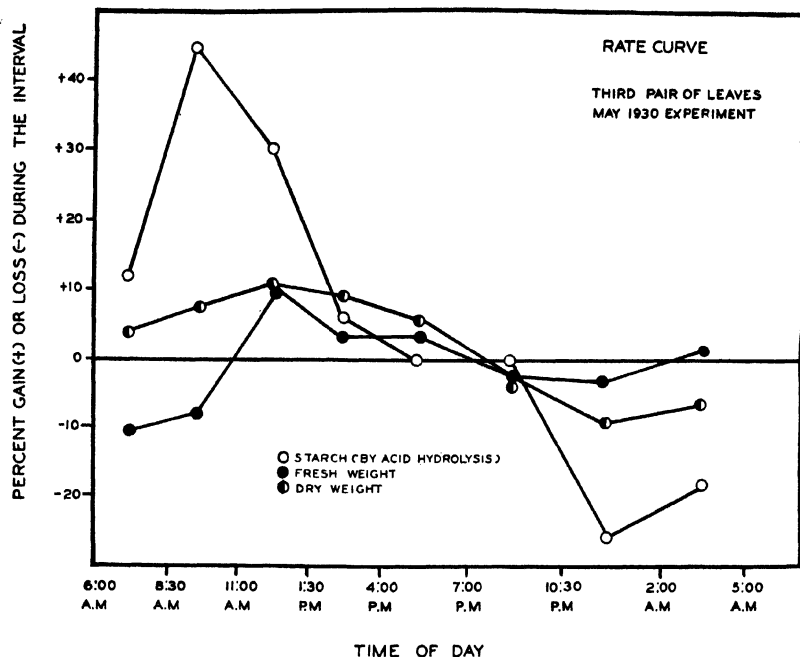
TABLE 7 B. *Percentage Changes and Relative Amounts Present at Intervals, as Calculated from Data in Table 7 A*

Time Interval	Percent Gain (+) or Loss (-) During Each Interval *						Relative Amount Present at End of Period, Amount at 6:00 A.M. as 100					
	Tip Leaves		Leaves Below Tip		Third Pair		Tip Leaves		Leaves Below Tip		Third Pair	
	Fresh Wt.	Starch	Fresh Wt.	Starch	Dry Wt.	Water	Fresh Wt.	Starch	Fresh Wt.	Starch	Fresh Wt.	Dry Wt.
6:00 to 8:30 A.M.	-6.0	+12.2	-6.8	+11.8	+3.3	-12.1	94	+11.8	93	112	90	103
8:30 to 11:00 A.M.	+1.2	+29.4	+0.9	+44.4	+7.3	-2.1	95	+44.4	94	145	83	111
11:00 A.M. to 1:30 P.M.	+3.8	+30.4	+4.2	+30.0	+10.2	-0.5	99	+30.0	98	189	92	122
1:30 to 4:00 P.M.	+6.4	+21.0	+4.4	+6.2	+9.1	+2.1	105	+6.2	102	229	94	133
4:00 to 7:00 P.M.	+3.9	+8.0	+3.5	+8.0	+5.0	+2.9	109	+8.0	106	247	98	140
7:00 to 10:30 P.M.	+4.1	+2.7	+2.6	0	-2.4	-1.6	113	0	109	247	96	131
10:30 to 2:00 A.M.	-0.4	-8.2	-1.3	-26.0	-9.9	-1.9	113	-26.0	107	233	93	118
2:00 to 5:00 A.M.	+4.4	-28.8	+1.2	-18.6	-6.9	+2.1	118	-18.6	109	166	93	109

\* Percent change during each interval calculated on the amount present at the beginning of that period.

## Water Content

This was obtained in the 1930 experiment only for the third pair of leaves (second pair below tip, see table 7A, column 8, and table 7B, column 7). Losses of water were high until 11:00 A.M., at which time the water



TEXT FIG. 3. Rate curve; gain or loss during each period expressed as the percent of the amount present at the beginning of the period, and plotted at the middle of each period. All three curves for the third pair of leaves, *i.e.*, second pair below tip. *Salvia splendens* Ker.

content began to gain; but these leaves did not recover their original water content during the night as was the case in the 1929 experiment.

*Starch (Acid-hydrolyzable Polysaccharids)*

The data for the pair of leaves below the tip and for the third pair of leaves are shown in table 7A, columns 4 and 9, and in table 7B, columns 4, 8, 11, and 14. The percentage gains during each 2.5 hour period were again large in the forenoon, the gains being 30 to 44 per cent of the amount present at the beginning of each period. The results of the 1930 experiment differed from those of 1929 in the time during the night at which the losses began to take place. In the 1929 series this reduction in the amount of starch started in mid-afternoon (see text fig. 2), but in the 1930 series losses in starch did not begin until about midnight (see text fig. 2); the

starch values remained nearly constant from about 4:00 P.M. until about 10:30 P.M.

### *Insoluble Nitrogen*

In the 1930 as in the 1929 experiments the data show very little change in the amount of nitrogen in the insoluble portion. The absolute amounts in the samples at the beginning and at the end of each period fail to show any clear gains or losses that can not be accounted for as experimental errors, and the percentage of the fresh weight showed nearly the same values throughout the day. Here again the values tend to be slightly higher during the middle of the day but the difference is not great enough to be conclusive.

### *Sugar*

The preliminary experiments in 1929 indicated that *Salvia* leaves were very low in sugar, this constituent being about 0.1 to 0.3 percent of the fresh weight. In the 1930 experiments an attempt was made to measure the sugar change during the day by means of the Somogyi (11) modification of the Shaffer and Hartman (10) method. This is applicable to amounts of sugar varying from zero to two milligrams in a five cc. sample. The sugar values obtained in the 1930 experiments for the various samples throughout the day are shown in table 8 which gives not only the absolute

TABLE 8. *Diurnal Variation of Sugar in Leaves of Salvia May 1930 Experiment*

Time of Day	Tip Leaves				Third Pair			
	Reducing Sugar		Sucrose *		Reducing Sugar		Sucrose *	
	Total in 20 Leaves mg.	% of Fresh Weight	Total in 20 Leaves mg.	% of Fresh Weight	Total in 15 Leaves mg.	% of Fresh Weight	Total in 15 Leaves mg.	% of Fresh Weight
6:00 A.M. . . . .	7.2	0.17	4.3	0.10	8.6	0.11	7.8	0.09
8:30 A.M. . . . .	8.5	0.21	6.3	0.16	9.6	0.13	10.0	0.13
8:30 A.M. . . . .	9.9	0.24	7.5	0.15	12.5	0.14	11.1	0.12
11:00 A.M. . . . .	10.3	0.25	9.0	0.22	16.1	0.18	13.8	0.15
11:00 A.M. . . . .	9.9	0.27	8.1	0.22	13.4	0.17	13.2	0.17
1:30 P.M. . . . .	8.6	0.23	7.8	0.20	12.5	0.14	9.6	0.12
1:30 P.M. . . . .	8.8	0.24	7.1	0.20	13.7	0.16	13.2	0.16
4:00 P.M. . . . .	8.5	0.24	9.2	0.24	12.1	0.14	14.2	0.16
4:00 P.M. . . . .	7.0	0.22	9.4	0.29	12.6	0.15	13.6	0.16
7:00 P.M. . . . .	6.9	0.21	10.6	0.32	12.6	0.15	15.5	0.18
7:00 P.M. . . . .	7.4	0.21	9.9	0.28	8.2	0.12	14.0	0.18
10:30 P.M. . . . .	7.3	0.20	7.4	0.20	10.1	0.14	13.0	0.17
10:30 P.M. . . . .	6.5	0.18	9.7	0.26	8.3	0.11	14.4	0.18
2:00 A.M. . . . .	6.0	0.16	6.5	0.18	7.4	0.10	9.1	0.12
2:00 A.M. . . . .	6.7	0.18	6.3	0.17	8.8	0.11	13.8	0.16
5:00 A.M. . . . .	7.1	0.18	4.3	0.11	8.9	0.11	7.8	0.09

\* By acid inversion in the cold, see 1, p. 95.

amounts in the entire sample but also the fresh weight percentages. It is seen that only small changes occurred. The reducing sugar values gave increases up to about 11:00 A.M. and then fell off toward the next morning. The sucrose values are subject to greater error since they are arrived at by means of the difference of two measurements; but, as a whole, low values were obtained in the early morning samples, with higher values about 4:00 P.M.

It may be questioned whether these observed differences are real, in view of the small amounts present and the large percentage error involved in their determination. The percentage of the fresh weight varied only between the limits of 0.11 percent and 0.27 percent for the reducing sugar, and between 0.09 percent and 0.32 percent for the sucrose. Compared, therefore, with the changes that were observed in fresh weight, dry weight, starch, etc., the sugars have shown very little fluctuations during the day.

It should be stated that these sugar determinations were made with uncleared solutions and represent, therefore, not merely sugar but all other substances that reduce copper under these conditions. It was not found feasible to clear with lead acetate, since the volume of liquid available for the test was small and the losses in amounts of liquid resulting from the procedures in leading and deleading were large. Preliminary tests indicated that leading and deleading decreased the apparent sugar content by about one-fifth or one-fourth.

#### Dry Weight Increases per Square Meter of Leaf Surface per Hour

Time was not available for taking the leaf areas of all the samples in these series of measurements. But, to permit a comparison of these measurements with previous work on diurnal changes in which the results were always expressed on the leaf area basis, the general relation between the leaf weight and area was established. Thus, in table 9 are shown the

TABLE 9. *Relation Between Fresh Weight and Area of Salvia Leaves*

Fresh Weight of Leaf, grams	Area in sq. cm.	Fresh Weight of Leaf, grams	Area in sq. cm.
0.86	19.8	0.30	8.2
0.60	13.7	0.08	2.7
0.38	9.9	0.13	3.9
0.27	8.2	0.30	7.8
0.16	5.7	0.27	8.4
0.17	5.3	0.48	12.6
0.52	13.0	0.37	10.7
0.43	11.4	0.16	5.3
0.52	13.7	0.22	6.9
0.68	19.0	0.46	12.0

NOTE: The weights and areas of leaves were recorded in the above table in the order in which the measurements were made. From this table a graph (not shown in this paper) was prepared, giving the relation between weight and area. From this graph the average areas of the leaves of the various samples were calculated for use in table 10. Small leaves have a greater leaf area per fresh weight than large leaves, e.g., leaves that were 0.1 gram in weight gave areas of about 3.1 sq. cm. while those with weights of 0.3 g. and 0.5 g. gave areas of about 8.3 and 13.0 sq. cm., respectively.

fresh weights and leaf areas of 20 *Salvia* leaves of various sizes. From these measurements a graph (not shown) was prepared from which the average leaf weights in the various samples were translated into average leaf areas, at least with fair accuracy. The corresponding gain in dry weight per leaf area during each interval was calculated for the leaves below the tip in the 1929 experiment, and for the third pair of leaves in both years. The results are shown in table 10 which shows the gain in grams per square

TABLE 10. *Dry Weight Increase per Square Meter per Hour*

April 1929 Experiment			May 1930 Experiment	
Time Interval	Leaves Below Tip g. per sq. m. per hr.	Third Pair g. per sq. m. per hr.	Time Interval	Third Pair g. per sq. m. per hr.
5:30 to 8:00 A.M. ....	0.50	0.33	6:00 to 8:30 A.M. ....	0.66
8:00 to 10:30 A.M. ....	1.16	2.78	8:30 to 11:00 A.M. ....	1.58
10:30 to 1:00 P.M. ....	2.68	1.94	11:00 to 1:30 P.M. ....	2.17
1:00 to 3:30 P.M. ....	1.09	1.00	1:30 to 4:00 P.M. ....	2.28
3:30 to 7:00 P.M. ....	0.39	0.30	4:00 to 7:00 P.M. ....	1.05
Average . . . . .	1.16	1.27	Average . . . . .	1.54

meter of leaf area per hour during each interval in which gains in dry weight were made. It is seen that a series of values was obtained showing how the rate changed from one period to another. Thus, in the 1929 experiment, with the leaves below the tip, the successive gains were 0.5 gram per square meter per hour during the period from 5:30 to 8:00 A.M., 1.16 from 8:00 to 10:00 A.M., 2.68 from 10:30 A.M. to 1:00 P.M., 1.09 from 1:00 to 3:30 P.M., and 0.39 from 3:30 to 7:00 P.M. Thereafter the dry weight decreased. It is interesting to compare the results in the two different years, the maximum rate of gain occurring later in the day in the 1930 than in the 1929 experiments; e.g., for the third pair of leaves the highest rate was between 8:00 and 10:30 A.M. in 1929, and between 1:30 and 4:00 in the 1930 experiments.

We may compare these values with previous measurements of dry weight increase. Kostytschew (8, p. 177) gives the amounts per square meter per hour for different species, and in his list the values range from 1.00 to 2.37. The bottom line in table 10 shows that the average values in the present experiments (1.16 to 1.54 g. per sq. m. per hr.) come within the range of the Kostytschew values.

The gains in dry weight per leaf area are usually given as average values over a considerable period of time, often for a ten hour period, but the details in table 10 are of much greater interest, since they show not only the average over a considerable period but also show the values for each interval. It is seen that the rate over a short interval may be more than twice the average rate over a long period; and probably if suitable

conditions as to starch depletion before the start of the experiment were provided, even larger differences between gains during short exposures and average gains over a long period would be found.

#### DISCUSSION

It is likely that Sachs tested the opposite leaf possibility in connection with the early experiments on this subject, since he speaks (9, p. 7) of the use in some cases of opposite leaflets of compound leaves, but no detailed data on the point have yet come to the writer's notice; also Broocks (2) when dealing with plants "mit gefiederten Blättern (Bohne, Kartoffel)," used opposite leaflets, but there is no evidence that he regarded this as an improvement. Perhaps this method was tested and discarded by them for the reason that the symmetry error was found to be higher with opposite leaves than with opposite halves. They could not foresee, of course, that in later years there would be brought forward objections which would suggest the need of sacrificing accuracy in the sample weight in order to attain an advantage in another direction.

Even though it may be shown in the future that there is greater uniformity in opposite halves than in opposite leaves, we should not merely on that account condemn the twin-leaf method. It has an important advantage in that the errors arising from mutilation of the leaf are much reduced. We can not say at present that the cutting of the petiole has no effect at all upon the opposite leaf, but we can reasonably expect that the effect is small because of the small amount of tissue involved in cutting through the petiole, and because of the distance to the opposite leaf blade whose metabolism must be affected to bring about an error in the method.

The suggestion that the opposite halves of a leaf are more nearly in the same physiological condition than any two leaves upon the plant, does not appear to represent the situation correctly. This may be true when the leaf is intact, but when one half is cut away the physiological condition of the other half is so seriously disturbed that the previous advantage in this respect is no longer present.

There is a restriction in the number of kinds of plants with which the twin-leaf method can be used. Only those with opposite leaves or leaflets are available, and, of these, only those showing a sufficient uniformity in size and chemical composition for the purposes of the experiment. But it is believed that there are many such, and since in certain types of experiments the species to be used can be deliberately chosen, it is possible that the method can find a good field of usefulness.

#### SUMMARY

1. Because of the objections that have been made against Sachs' half-leaf method of measuring changes in leaves during a definite time interval, attention was turned to the possibility of using the pair of leaves of species



having opposite leaves or leaflets, the plan being to take one leaf of the pair at the beginning of the period and the other one at the end. It is suggested that this be called the "twin-leaf" method to distinguish it from the half-leaf method. An alternative name is the "opposite-leaf" method.

2. In order to determine the extent of the variation in opposite leaves, samples of single pairs and of composite samples including several leaves were taken, fresh and dry weights were obtained, and in some cases chemical analyses for various constituents were made.

3. Tests of different species showed favorable results with several, the error involved in the assumption that the weights of the opposite leaves were equal amounting in *Salvia splendens* to about five percent, which would represent an error of about one percent on a composite sample of 25 leaves and of about two percent on a sample of nine.

4. The method was then applied to the determination of diurnal changes in the leaf blades of *Salvia*. Samples were taken on April 3, 1929, at 5:30, 8:00, 10:30 A.M., 1:00, 3:30, 7:00, 11:00 P.M., and 4:00 A.M.; 30 leaves were taken at the beginning of each period and the opposite leaves in each pair at the end. Another series was carried through the 24 hour period on May 12, 1930, samples being taken at 6:00, 8:30, 11:00 A.M., 1:30, 4:00, 7:00, 10:30 P.M., 2:00 and 5:00 A.M., 15 to 20 leaves being taken at each period and all samples being made comparable by the use of opposite (twin) leaves. In both series leaves of three types were collected: tip leaves, leaves below the tip, and the third pair (*i.e.*, second pair of leaves below the tip). The three types of leaves were collected and analyzed separately.

5. The analytical data for the various samples at each period included fresh weight, dry weight, water content, starch, soluble solids, insoluble nitrogen, etc. The tables and graphs show the absolute amounts of material present in each sample, the percentage gain or loss during each period, and the relative amount of each constituent at any time with respect to the amount present in the first sample taken in the early morning.

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## OXYGEN REQUIREMENTS FOR ROOT GROWTH OF CUTTINGS IN WATER<sup>1</sup>

P. W. ZIMMERMAN

### INTRODUCTION

Those who attempt to review the literature on aeration as affecting plant growth find it varied and voluminous. Many reports are conflicting as might be expected in so large a field. Some phases of the subject, such as oxygen requirement for growth in liquid media, have not been extensively investigated. The present paper shows some of the effects of known amounts of dissolved oxygen on growth of roots from cuttings.

Livingston and Free (10), using sealed soil containers which could be auto-irrigated and aerated, concluded that plants vary in their requirements for oxygen, willow being a low and coleus a high oxygen type. Complete deprivation of oxygen caused sensitive species like coleus and heliotrope to wilt. The roots failed to take up water and the plants soon died. Cannon (3) in 1915 noted a relationship between moisture, aeration, and temperature as environmental factors which control the distribution of plants.

Free (8) found that buckwheat in culture solutions was not improved by aeration with air, oxygen, or nitrogen. It was not injured by nitrogen but was killed when aerated with carbon dioxide. Cannon (4) in 1925 showed that the requirements of cotton for oxygen varied with the temperature. Growth was normal at 21° C. when the air surrounding the roots had only 2.6 percent oxygen, while the plants in this same amount of oxygen but at 28° C. gave approximately one-fourth of normal growth. Corn at 18° C. or higher required more than 10 percent oxygen in the air surrounding the roots. When the growth rate of plants was normal for a given concentration of oxygen, the addition of more of this gas did not further increase growth. Emerson (7) found the subterranean systems of plants growing on floating bog mats to be very superficial and nearly all above the water. He thought that some of this superficial development might have been due to toxic materials in bog water, but that oxygen doubtless played a part. Bergman (1) observed that roots of land plants do not live under prolonged submergence. The roots soon die and new ones are developed from the

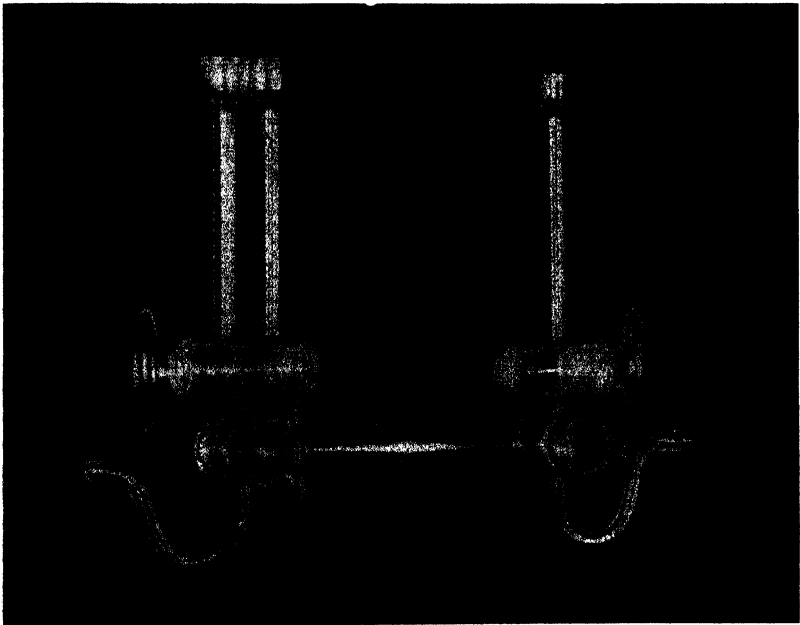
<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

stem near the surface of the water. When, however, the water was aerated the roots were able to endure submergence, though some retardation was noted. In nature the oxygen content of swamp water decreased from the *Carex* stage to the *Chamaedaphne-Andromeda* stage. Ecesis, he concludes, can occur only when the oxygen requirements are satisfied. Bergman (2) noted that the oxygen content of water where cranberry plants were submerged varied on cloudy and clear days. Shaded tubs containing submerged plants had less oxygen than controls in direct sunlight.

In 1921 Clements (5) published a monograph in which he summarized approximately 700 papers dealing with aeration and air content. With this publication at hand there is little need in this paper for further references to literature. The data reported herewith are primarily to show the effect of various concentrations of oxygen on the production of roots by cuttings.

#### METHODS

Cuttings were placed in water of different depths. The containers used were glass cylinders 9 inches in height by  $1\frac{1}{2}$  inches in diameter or large



TEXT FIG. 1. Apparatus used for micro-determination of oxygen in water.

test tubes 16 inches by 2 inches. A complete experiment usually consisted of aerated and not aerated cuttings in shallow, medium deep, and deep water. Aeration was accomplished by bubbling air or oxygen from cylinders

through the water. Five cc. samples of water were withdrawn from various depths and analyzed for oxygen.

The analyses<sup>2</sup> were made with a specially designed apparatus described by Thompson and Miller (12). A picture of this apparatus may be seen in text figure 1.

In a few cases oxidizing compounds such as potassium permanganate and hydrogen peroxid were added in different amounts to tap water in which the cuttings were grown. Additional amounts of these chemicals were added at regular intervals.

Tap water was used in most cases. When it was necessary to start with water that was low in oxygen the tap water was first boiled. In some cases, paraffin oil was placed over the surface of the water to decrease absorption of oxygen from the air.

Where light appeared to be a factor in controlling root development or oxygen content the tubes were either wrapped with black paper or placed in a dark room.

Any variations in the methods are described in connection with the report of results.

#### MATERIAL

*Salix pendula* (willow), *Forsythia intermedia*, rose (Dorothy Perkins), *Salvia splendens*, *Coleus Blumei*, *Hedera helix* (English ivy), *Lycopersicum esculentum* (Bonny Best tomato), *Ligustrum ovalifolium* (privet), *Philadelphus* sp., *Chrysanthemum* sp., *Prunus tomentosa*, *Portulaca oleracea* constituted the main types that were tested. Long cuttings were used so that they could be placed in deep or shallow water. Both leafy shoots and dormant leafless stems were tested in the course of a year.

#### RESULTS

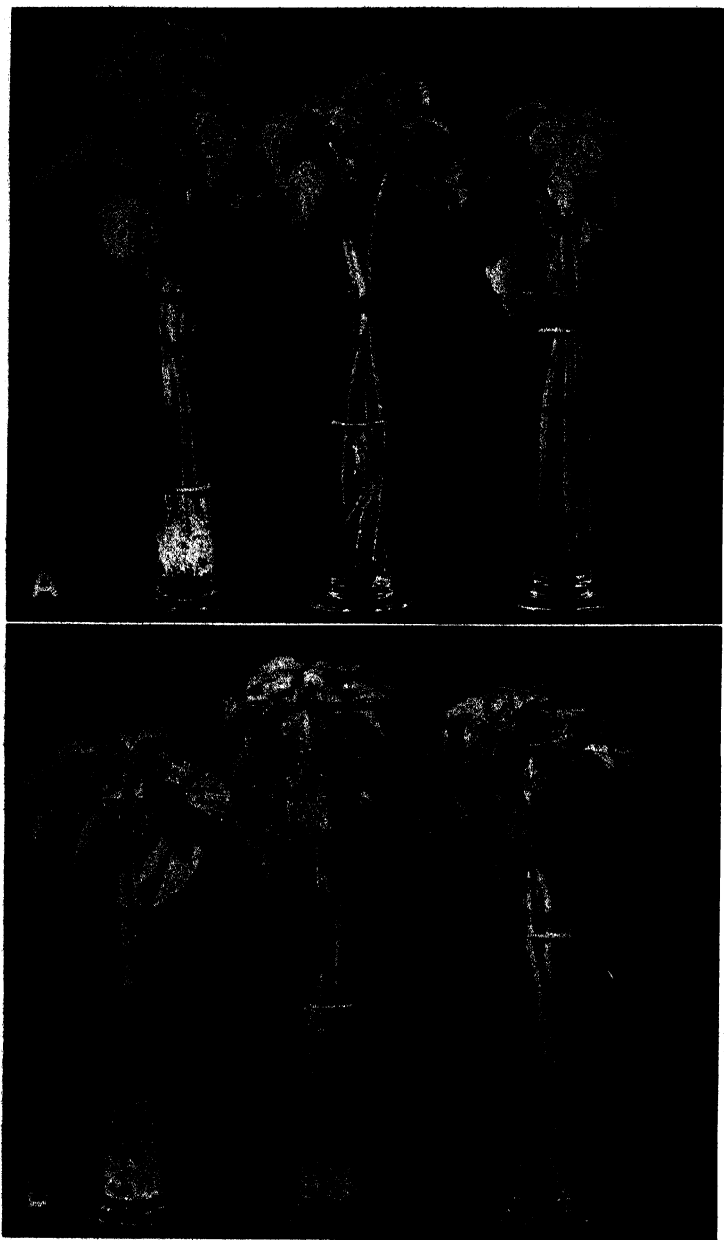
Four long *Salvia splendens* cuttings were placed in each of 12 cylinders so that the basal ends rested on the bottom and the leaves extended above the glass. The cylinders were then divided into four lots of three each for various treatments as shown by the following plan:

<sup>2</sup> The method of procedure up to the point of titration was well described by Thompson and Miller (12) and for that reason is omitted from this paper. The iodine liberated was titrated with N/500 sodium thiosulphate. According to Scott (11) 1 cc. of N/40 sodium thiosulphate is equivalent to 0.2 milligram oxygen by weight or 0.1395 cc. oxygen by volume under standard conditions. Then 1 cc. of N/500 sodium thiosulphate would be the equivalent of .000016 g. of oxygen. On a 5 cc. sample, which represents the volume analyzed with our apparatus, 1 cc. of N/500 sodium thiosulphate =  $.000016 \times 100/5$  or .00032 percent of oxygen or 3.2 p.p.m. To calculate the parts per million of oxygen in the 5 cc. sample multiply the number of cc. of sodium thiosulphate used in titration by the factor 3.2.

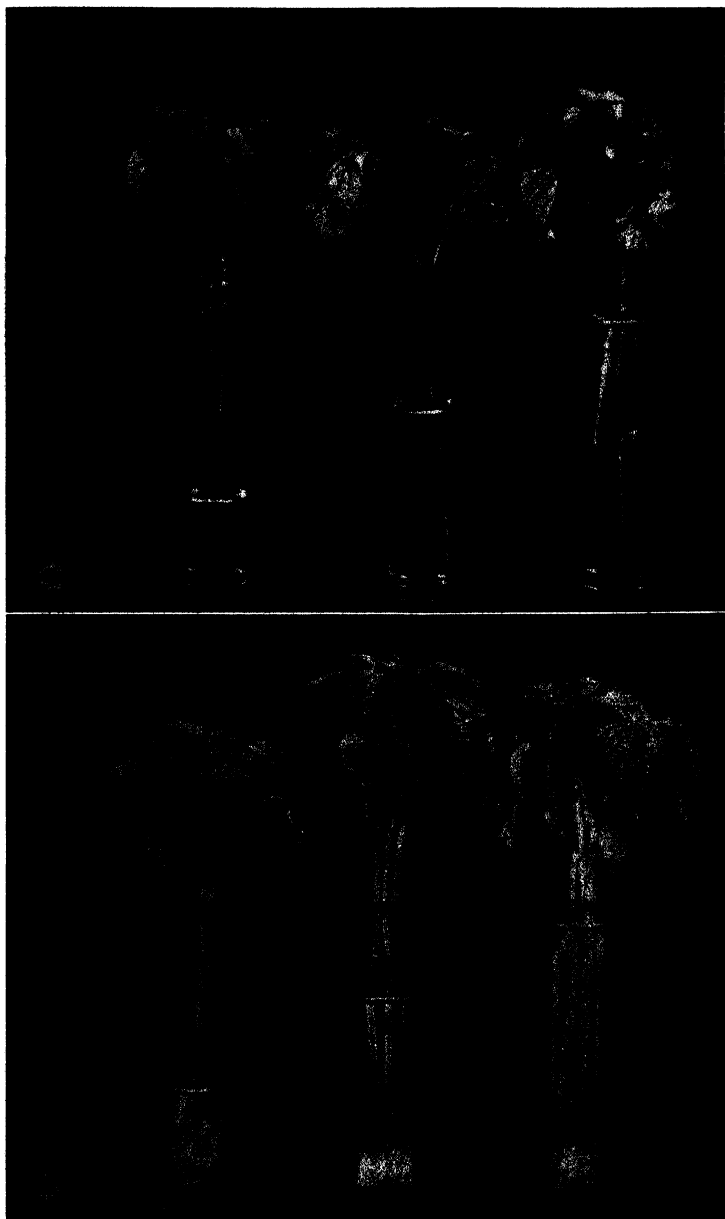
Lot	Depth of Water Indicated in Inches	Treatment
Lot A, cylinder 1.....	1 $\frac{1}{2}$	Tap water not aerated
Lot A, cylinder 2.....	4 $\frac{1}{2}$	
Lot A, cylinder 3.....	7	
Lot B, cylinder 1.....	1 $\frac{1}{2}$	Tap water aerated with oxygen
Lot B, cylinder 2.....	4 $\frac{1}{2}$	
Lot B, cylinder 3.....	7	
Lot C, cylinder 1.....	1 $\frac{1}{2}$	Water was boiled then oiled with paraffin oil to prevent oxygen absorption from the air
Lot C, cylinder 2.....	4 $\frac{1}{2}$	
Lot C, cylinder 3.....	7	
Lot D, cylinder 1.....	1 $\frac{1}{2}$	Water was boiled then oiled with paraffin oil and aerated with oxygen
Lot D, cylinder 2.....	4 $\frac{1}{2}$	
Lot D, cylinder 3.....	7	

The water used in two lots was boiled so as to lower its oxygen content at the beginning of the experiment. In two lots the water was aerated with oxygen from a tank through glass tubes extending to the bottom of the water column. Also in two lots paraffin oil was placed on the surface of the water to decrease the absorption of oxygen from the air. The water levels were kept constant by means of siphons from reservoirs. The results of the experiment are shown in text figures 2 and 3. Rooting occurred in lot A at the base of the cutting in shallow tap water, but in the deeper water roots appeared some distance above the base near the surface of the water. Cuttings in the deepest water did not root. Lot B which was the same except that the water was aerated showed rooting at the base of all the cuttings even in the deepest water. Lot C in water which had been boiled and oiled produced no roots, but Lot D which had water that had been boiled, oiled, and then aerated produced roots at the base in all depths of water. Where aeration was maintained salvia cuttings rooted regularly at the base, whereas rooting occurred above the base in four inches of non-aerated water. At the time this experiment was in progress equipment for determining small quantities of oxygen in water was not available, but there was strong evidence that oxygen was the limiting factor. Accordingly, in later experiments a method was worked out whereby 5 cc. samples of water could be drawn out of the cylinders and analyzed quantitatively for oxygen. The method used was a modification of the Winkler (13) method with an apparatus described by Thompson and Miller (12). With this method it was possible to withdraw and analyze a sample from any point along the stem with very little mixing of the water.

In order to get quantitative measurements of minimum oxygen requirements for root growth, some cuttings were selected from plant types like willow which have the capacity to root at various places along the stem. These cuttings were grown in 16" glass tubes as shown in text figure 4. The response was somewhat similar to that of salvia in that there was a



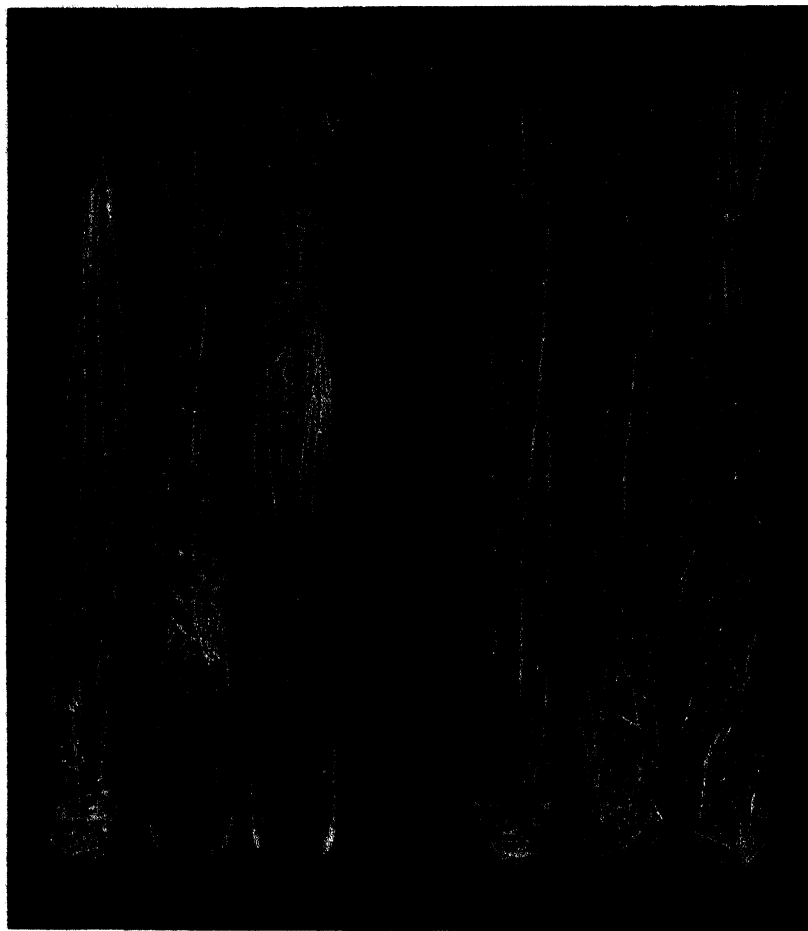
TEXT FIG. 2. *Salvia* cuttings in water from August 2, 1929 to August 24, 1929. A, cuttings in tap water not aerated; B, cuttings in tap water aerated with oxygen.



TEXT FIG. 3. *Salvia* cuttings in water from August 2, 1929 to August 24, 1929. *C*, cuttings in water that was boiled to remove the oxygen and then oiled with paraffin oil to decrease oxygen absorption; *D*, cuttings in water that was boiled, then oiled and aerated.



tendency for roots to form near the surface of non-aerated deep water and at the base of the aerated cuttings. This peculiar response in non-aerated tubes furnished an opportunity to determine the amount of oxygen where roots grew well, or poorly, or not at all. The results of analyses are reported in table 1. Text figure 4 shows the root response in different depths of



TEXT FIG. 4. Willow cuttings started October 24, 1929 and photographed November 16, 1929. The glass tubes were 16" long by 2" in diameter. The water depths were 2", 8", and 15".

Left, three tubes not aerated; right, three tubes aerated with oxygen from a commercial cylinder of the gas.

aerated and non-aerated water. Roots grew near the surface of the water in the non-aerated tubes. At the time the experiment was started tap water

had approximately eight parts of oxygen per one million parts of water, but eight days later when roots were starting the oxygen in the non-aerated shallow water had been reduced to 3.2 p.p.m. At the bottom of an 8" column of water the reading was 0.6 p.p.m. and at the bottom of a 15" column no oxygen was detected and no roots had grown. The aerated series showed at the same time rooting and approximately 18 parts of oxygen per million of water at all points in the tubes.

TABLE 1. *Response of Salix pendula (willow) cuttings approximately 18" long taken Oct. 24, 1929 and placed in test tubes (16" X 2") containing three different depths of water to determine the oxygen requirements for root growth*

Treatment and Date of Analyses	Depth of Water Column, in Inches	Point Below Surface from Which Sample Was Drawn, in Inches	Oxygen p.p.m.	Length of Roots in Inches
<b>Series A</b>				
Not aerated				
Analyses made 11/2				
Tube 1 .....	2	2	3.2	Starting
Tube 2 .....	8	8	0.64	0
Tube 3 .....	15	15	0.00	0
<b>Series B</b>				
Aerated with Oxygen				
Analyses made 11/2				
Tube 1 .....	2	2	18.7	Starting
Tube 2 .....	8	8	19.2	Starting
Tube 3 .....	15	15	18.7	Starting
<b>Series A</b>				
Not aerated				
Analyses made 11/6				
Tube 1 .....	2	1	6.6	2
Tube 2 .....	8	1	0.5	1
Tube 2 .....	8	8	0.1	0
Tube 3 .....	15	1	0.6	1
Tube 3 .....	15	7	0.3	0
Tube 3 .....	15	15	0.2	0
<b>Series B *</b>				
Aerated with Oxygen				
				About 2 in all depths

\* Analyses were not made throughout on the aerated series because they always showed much more oxygen (approximately 20 p.p.m.) than is required for root growth.

Thirteen days after the experiment had been started, roots were growing near the surface of the water in all the non-aerated tubes. In the shallow water, however, the roots were two inches long with the oxygen at 6.6 p.p.m. as contrasted with one-fourth inch roots in the deep water, where the oxygen had been reduced to 0.6 p.p.m. (see table 1). In the aerated series the roots were approximately two inches in length and the oxygen content was approximately 18 p.p.m. throughout the tubes. Though the roots grew throughout the deep aerated water there was a tendency for the cuttings to exhibit some polarity by having the largest roots near the base. Polarity was disturbed in the deep water, non-aerated series, due to the very low oxygen supply at the base of the cuttings.

TABLE 2. Variation of oxygen supply in water of aerated and non-aerated lots of *Salix pendula* (willow) cuttings. Experiment started 11/22/29

Treatment and Date of Analyses	Depth of Water Column, in Inches	Point Below Surface from Which Sample Was Taken, in Inches	Oxygen p.p.m.	Length of Roots, in Inches
<b>Series C</b>				
Not aerated				
Analyzed 11/26/29				
Tube 1 .....	3	3	3.4	0
Tube 2 .....	8	8	1.34	0
Tube 3 .....	15	15	0.64	0
<b>Series D</b>				
Aerated with oxygen				
Analyzed 11/26/29				
Tube 1 .....	3	3	18.5	0
Tube 2 .....	8	8	18.0	0
Tube 3 .....	15	15	15.0	0
<b>Series C</b>				
Not aerated				
Analyzed 12/6/29				
Tube 1 .....	3	3	4.8	$\frac{1}{2}$
Tube 2 .....	8	1	0.96	0
Tube 2 .....	8	8	0.64	0
Tube 3 .....	15	15	0.3	0
<b>Series D</b>				
Aerated with oxygen				
Analyzed 12/6/29				
Tube 1 .....	3	3	19.6	$1\frac{1}{2}$
Tube 2 .....	8	8	18.0	1
Tube 3 .....	15	15	18.5	1
<b>Series C</b>				
Not aerated				
Analyzed 12/11/29				
Tube 1 .....	3	3	5.08	1
Tube 2 .....	8	4	1.44	$\frac{1}{2}$
Tube 2 .....	8	8	0.64	0
Tube 3 .....	15	2	0.16	0
Tube 3 .....	15	15	0.2	0
<b>Series D *</b>				
Aerated with oxygen				
			All aerated tubes were high in oxygen therefore they were not always tested	Roots in all tubes were 2 to 3
<b>Series C</b>				
Not aerated				
Analyzed 12/16 to 12/19/29				
Tube 1 .....	3	3	1.79	2+
Tube 2 .....	8	1	1.18	1
Tube 2 .....	8	8	1.05	1
Tube 3 .....	15	1	0.89	Roots starting
Tube 3 .....	15	7	0.8	Roots starting
Tube 3 .....	15	15	0.416	Roots starting
<b>Series D *</b>				
Aerated with oxygen *				
				3 to 4

\* Series D omitted because oxygen was high throughout and roots were large (see text fig. 4.)

In another series of experiments with *Salix pendula*, the cuttings in non-aerated water failed to deplete the oxygen supply and as a result roots grew more or less uniformly along the stems from the surface of the water to the base of the cuttings. The oxygen supply varied more than could be accounted for, first running low and then high toward the end of the experiment (see table 2). The tubes not having been wrapped with black paper, there is a possibility that the green stems in light caused an increase of oxygen through photosynthesis. Also, green algae developed in some of the tubes and might have been responsible for keeping the oxygen high.

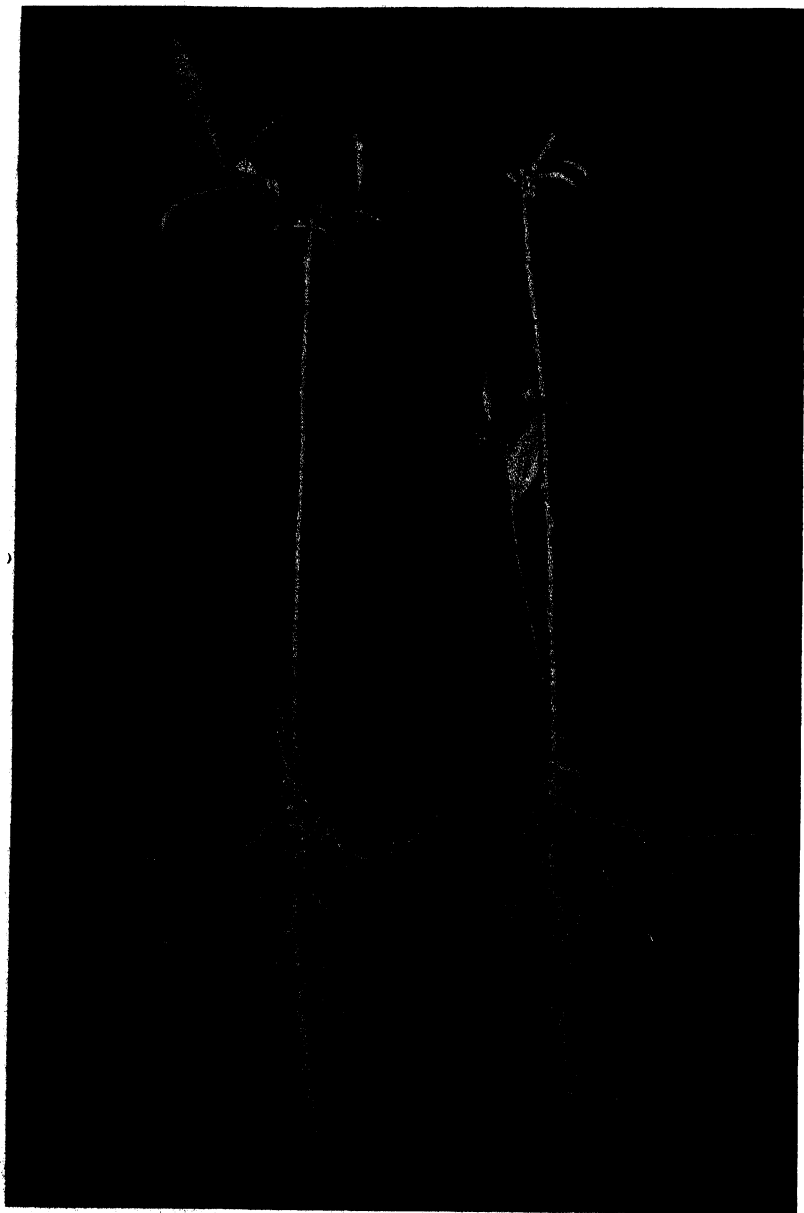
The aerated lot of this series showed two striking differences from the non-aerated. The roots in aerated water were produced near the base of the cuttings and there was very little development of excrescences from the lenticels (text fig. 5). Hahn, Hartley, and Rhodes (9) stated that hypertrophied lenticels were produced on conifer roots in various types of soil in the presence of excessive moisture. Contrary to previous views that such hypertrophies are due to increased sap pressure, these authors believe that such excrescences may also be due to oxygen deficiency. There is a close relationship between the available oxygen supply and the development of hypertrophied lenticels on willow stems. The greatest development yet found was at a place where the supply was approximately one part of oxygen to one million parts of water. The development of these excrescences then diminished toward the base of the cuttings where the oxygen was nearly exhausted (text fig. 5). Aerated willow cuttings produced roots without at the same time producing hypertrophied lenticels, but when the aeration was stopped for a few days excrescences developed rapidly.

Running water through tubes had essentially the same effect on root growth as aerating the water. This result might be expected since the oxygen in tap water was usually eight to ten parts per million of water which is more than has been found necessary for growth of willow roots.

Oxygen requirement varied with the species. *Hedera helix* (English ivy) was grown in aerated and non-aerated water. Table 3 shows the results of

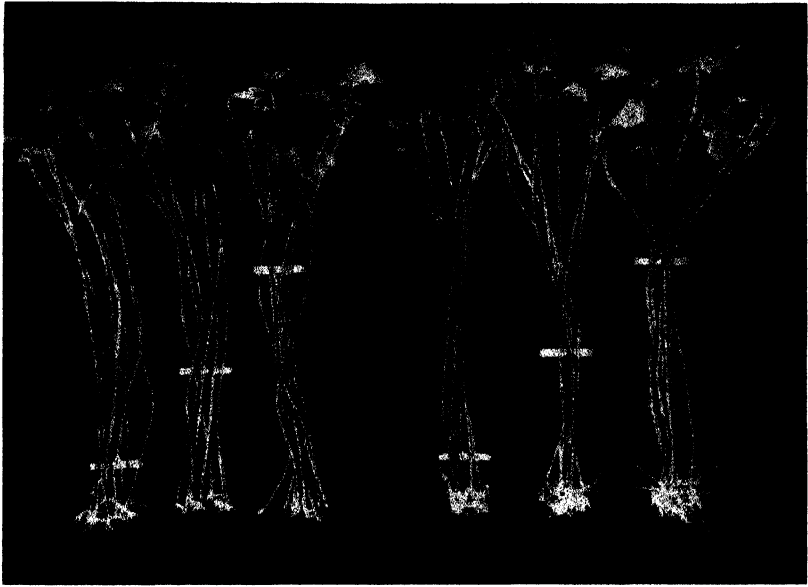
TABLE 3. *Hedera helix* (English ivy) in aerated and non-aerated water. The experiments started Nov. 12, 1929 and the analyses were made Dec. 9, 1929. See text figure 6

Treatment	Point Below Surface at Which Sample Was Taken, in Inches	Oxygen p.p.m.	Approximate Root Length in Inches
Not aerated			
Tube 1 . . . . .	1½	4.5	2
Tube 2 . . . . .	4	4.0	1
Tube 3 . . . . .	7	1.8	½
Aerated with oxygen			
Tube 1 . . . . .	1½	22.0	2 to 3
Tube 2 . . . . .	4	22.4	2 to 3½
Tube 3 . . . . .	7	20.1	2 to 4



TEXT FIG. 5. Willow cutting in non-aerated water. Note the hypertrophied lenticels near the surface of the water and how they decrease in size to the base of the cutting where the oxygen supply was low (0.1 to 0.3 parts per million of water).

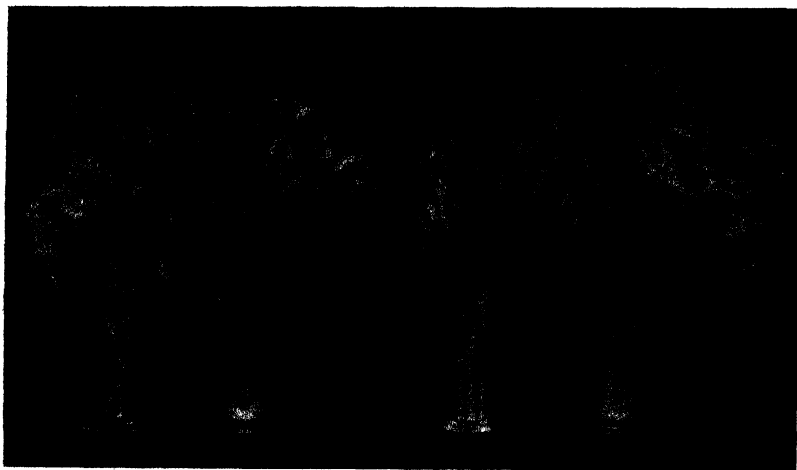
water analyses 30 days after the experiment was started, and text figure 6 shows the appearance of the roots. The stems of the cuttings were green and it is likely that through the photosynthetic process the oxygen content



TEXT FIG. 6. Aerated and non-aerated English ivy cuttings in three depths of water. The white line across the stems indicates the height of the water column. The shallow cuttings were in one and one-half inches of water, the medium four inches, and the deep seven inches. The experiment started November 12, 1929 and the photograph was made December 13, 1929, a total of 32 days. Left, not aerated; right, aerated.

was kept higher in the non-aerated tubes than would have been true for darkened cylinders. But even though the oxygen was as high as four parts per million in the non-aerated four-inch column of water the roots were much inferior to those of similar cuttings in aerated water. The specimens in seven inches of non-aerated water produced roots only one-eighth of an inch in length though the oxygen was 1.8 parts per million, which would be sufficient for good root growth of willow cuttings. The results of this experiment indicate that the rate of growth of ivy roots varies within limits according to the oxygen supply. With 1.8 p.p.m. of oxygen they made one-eighth of an inch of growth in contrast to three to four inches of growth in aerated water where the oxygen was 20 p.p.m. It is not likely that ivy would be benefited from supplying more than ten parts of oxygen per million of water where other factors are not limiting. The difference between the root growth of the shallow groups is only slight while the oxygen content ranges from 4.5 p.p.m. in the non-aerated to 20.1 p.p.m. in the aerated.

The oxygen supply in non-aerated water in which *Lycopersicum esculentum* (tomato) cuttings were kept varied with the depth of the water and also with exposure to light. Since the oxygen was never depleted in the deep water tubes, it is likely that the green tissue gave off oxygen through photosynthesis, thus aerating the water. Table 4 shows that several sets of tomato cuttings which had black paper wrapped around the containers nearly exhausted the oxygen supply whereas those that were not wrapped had four to six parts of oxygen per million of water throughout the time the experiment continued. The stems of tomato cuttings in deep water disintegrated readily when the container was wrapped and not aerated (text fig. 7).



TEXT FIG. 7. Tomato cuttings in 8 inches of water to find combined effect light and aeration vs. dark and aeration. Left to right (1) control in light, (2) aerated with oxygen in light, (3) control where the cylinder was wrapped with black paper, (4) cylinder wrapped with black paper and then aerated.

Note that no. 1 though rooting near the surface of the water instead of at the base, is in good condition while no. 3 treated like no. 1 except that cylinder was wrapped with black paper, is in bad condition. The cause of deterioration was due to a deficiency in oxygen.

Table 5 gives further proof that the green stems in light have an aerating effect on the water. When the water was oiled to decrease oxygen absorption from the air and wrapped to shut out the light, the final analysis showed the oxygen to be only 0.48 p.p.m., as compared to 6.7 p.p.m. for the check. The table further shows that oil decreased the oxygen absorption from the air. The oiled water in light contained 3.2 p.p.m. as contrasted with 6.7 p.p.m. for the check where the water had not been oiled.

Callus formation varied with depth of the tissue under water. Dorothy Perkins rose cuttings formed both roots and callus in three inches of water

TABLE 4. *The results of analyses to show the amount of oxygen in water in which tomato cuttings were grown. The purpose of the experiment was to determine whether light had any effect on the oxygen content of the water in which the green stems were immersed. Each set contained three tomato cuttings in a large glass tube filled with water*

Treatment	Set No.	Date Started	Date Analyzed	Point Below Surface at Which Sample Was Taken, in Inches	Oxygen p.p.m.	Root Length, in Inches
In direct light in the greenhouse	1	11-22	11-25	14	4.28	0
	2	11-22	11-25	8	6.2	starting
	1	11-22	11-26	14	5.5	starting
	2	11-22	11-26	8	6.4	$\frac{1}{8}$
	1	11-22	11-27	14	5.12	$\frac{1}{2}$
	2	11-22	11-27	8	6.2	$\frac{1}{2}$
	1	11-22	11-29	14	6.56	1 to 2*
	2	11-22	11-29	8	4.32	1 to 1 $\frac{1}{2}$
	5	11-29	12-2	9	4.35†	0
	5	11-29	12-3	9	6.78	$\frac{1}{2}$
	5	11-29	12-4	9	6.72	$\frac{3}{4}$
	7	12-4	12-9	2	4.12	1
	8	12-4	12-9	2	5.44	1 $\frac{1}{2}$
Tubes covered with black paper	3	11-22	11-25	14	1.41	0
	4	11-22	11-25	8	3.84	starting
	3	11-22	11-26	14	1.47	0
	4	11-22	11-26	8	4.8	$\frac{1}{8}$
	3	11-22	11-27	14	0.57	0
	4	11-22	11-27	8	2.36	$\frac{1}{2}$
	3	11-22	11-29	14	0.7	0
	4	11-22	11-29	8	2.04	1 to 2*
	6	11-29	12-2	9	1.2	0
	6	11-29	12-3	9	2.84	0
	6	11-29	12-4	9	3.2	roots starting
	8	12-4	12-9	2	1.7	1 $\frac{1}{2}$
	9	12-4	12-9	2	2.6	1 $\frac{1}{2}$

\* Roots in aerated water were 3" to 4" in length.

† Cloudy day.



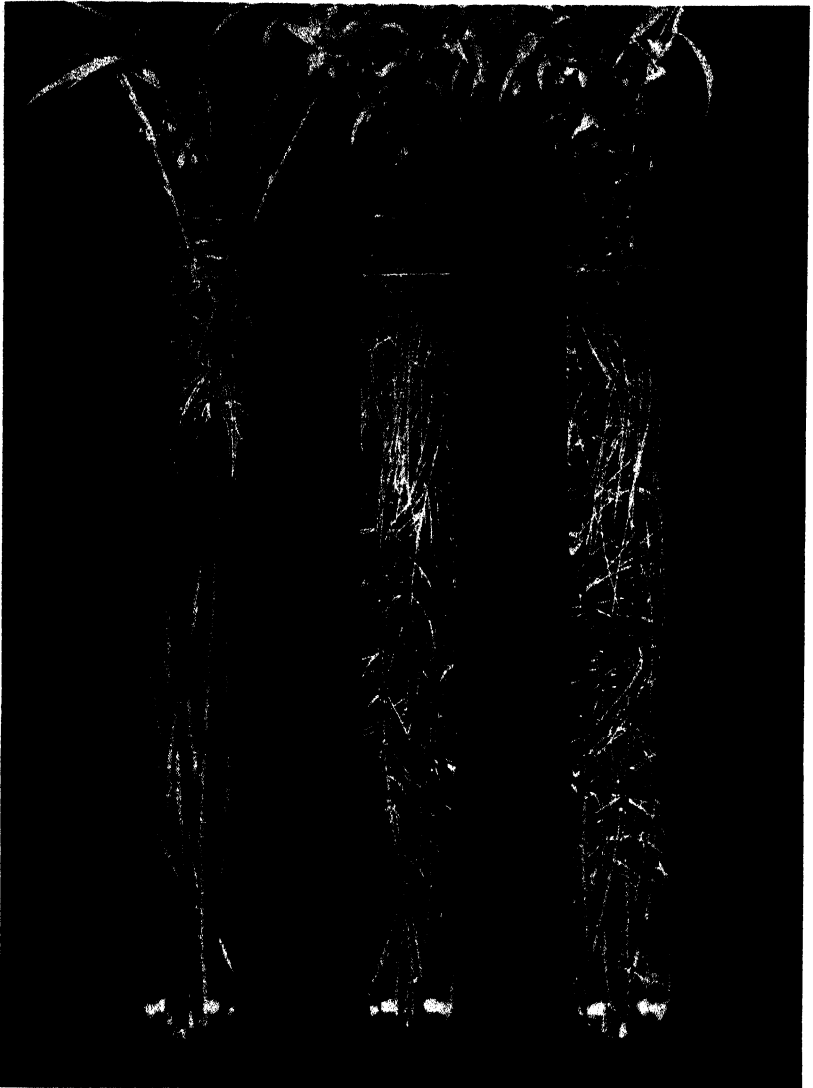
TABLE 5. *Oxygen content of water in which tomato cuttings were growing. The purpose of the experiment was to determine the effect of light on the oxygen supply of the water in which green stems were kept. The method was the same as described for Table 4, except that in some cases paraffin oil was placed on the surface of the water to decrease oxygen absorption from the air. The water samples analyzed were taken from approximately 9" below the surface*

Treatment	Set No.	Date Started	Date Analyzed	Oxygen p.p.m.
Check in light.....	1	11-29	12-2	4.35*
	1	11-29	12-3	6.78
	1	11-29	12-4	6.72
Oiled but in light.....	2	11-29	12-2	4.03
	2	11-29	12-3	4.8
	2	11-29	12-4	3.2
Tubes wrapped with black paper.....	3	11-29	12-2	1.2
	3	11-29	12-3	2.8
	3	11-29	12-4	3.2
Oiled and wrapped with black paper.....	4	11-29	12-2	0.9
	4	11-29	12-3	0.38
	4	11-29	12-4	0.48
Oiled and aerated with oxygen.....	5	11-29	12-2	23.0
	5	11-29	12-3	24.0
	5	11-29	12-4	20.0

\* Cloudy day

but not in 8 inches of water. No analyses were made but it seems fair to assume that there were about 4 or 5 p.p.m. of oxygen in the shallow water and much less in the deep water.

Of the oxidizing agents, hydrogen peroxid and potassium permanganate have been most effective. Text figure 8 shows the effect on production of roots of different concentrations of hydrogen peroxid in tap water. Evidently the upper limits had not been reached with three cubic centimeters of hydrogen peroxid per week. The analysis indicated as high as 17 parts of oxygen per million of water where three cubic centimeters per week had been used, but the supply varied from the time the new supply was added, becoming low by the end of the week. As shown by the photograph (text fig. 8) the place where roots grew differed greatly from that of aerated cuttings (text fig. 4). In aerated water, the roots grew best at the base but in water containing hydrogen peroxid the best roots grew near the surface of the water. This response was probably due to the low supply of oxygen maintained by the hydrogen peroxid toward the end of the period. Analyses of the water shortly after the hydrogen peroxid was added indicated oxygen as high as 50 p.p.m., but after six days the supply was about that of the control tubes.



TEXT FIG. 8. Three sets of willow cuttings showing the effects of three different concentrations of hydrogen peroxid in tap water. The experiment was started December 21 1929, and the results photographed on January 1, 1930.

Left, tube given one cc. of hydrogen peroxid each week.

Middle, tube given two cc. of hydrogen peroxid each week.

Right, tube given three cc. of hydrogen peroxid each week.

Roots from willow cuttings were produced along the entire length of stems which were immersed in weak solutions of potassium permanganate. Cuttings so treated differed from those in water aerated with oxygen in that those aerated produced most roots at the basal end of the stems, and a decreasing number toward the surface of the water. Polarity was not so much in evidence where permanganate was substituted for aeration. Quantitative measurements for free oxygen in the permanganate solution were not made because the permanganate interferes with the operation of one of the reagents (manganese sulfate) of the Winkler method. It is not clear just how an oxidizing agent like permanganate can substitute for the oxygen requirements of cuttings in water. Curtis, (6, p. 97) states that it is generally well known that when potassium permanganate comes in contact with organic matter, manganese dioxid is precipitated and oxygen is liberated. There is some doubt as to whether free molecular oxygen is actually liberated in the water, but however that may be, permanganate in some way partially substitutes for aeration. In the case of hydrogen peroxid probably free oxygen is actually liberated in the water.

#### DISCUSSION

Several striking responses have occurred in the course of these experiments. *Salvia* cuttings commonly show strong polarity and have no tendency to root up along the stem if they are properly aerated, but if the oxygen content of the water is lower than the minimum required for this species then roots tend to grow near the surface of the water. The oxygen content decreases from the surface down to the bottom of the water column thus accounting for poor root growth at the base of the stems. If a large number of cuttings are placed in a small volume of deep water, they soon deplete it of oxygen and then show signs of wilting. Livingston and Free (10) found that coleus and heliotrope growing in soil would wilt readily if completely deprived of oxygen. *Salvia* cuttings wilted even though the stems were immersed in 15 inches of water. Aerated cuttings in deep water remained in good condition and formed roots at the basal end of the stems. Long tomato cuttings in deep water responded like *salvia*, and when in darkness the non-aerated stems disintegrated very readily. Even wrapping of the cylinders with black paper caused the cutting to wilt and the stems to decompose. A thin layer of paraffin oil on the water decreased the absorption of oxygen from the air and hastened the destruction of cuttings in wrapped cylinders. The green stems partially substituted for aeration when the light intensity was great enough. This was accomplished, presumably, through photosynthesis by the green stems under water. Bergman (2) noted that the oxygen content of water containing submerged cranberry plants was greater on clear than on cloudy days.

Aerated cuttings produced their best roots near the basal end of the stems while the roots of non-aerated cuttings were best near the surface of the

water. Even willow stems, which have the capacity to root along the stem, showed strong polarity if placed in aerated water. When the basal end of a stem had an inadequate oxygen supply, the functioning region moved up near the surface of the water where the oxygen content was highest. The basal end remained alive and resumed normal growth when properly aerated. This suggests that to maintain a correlation influence the cells must not only be alive but also have the conditions which permit of growth.

Hypertrophied lenticels were in some way associated with aeration. They were largest in willow stems that were submerged in non-aerated water. Aerated water inhibited development of these excrescences. In non-aerated water they were largest where the roots were best and decreased in size together with the roots down toward the base of the stem where the oxygen content was nearly depleted. Like the roots in non-aerated water, hypertrophied lenticels grew well when the oxygen was 1 to 2 p.p.m., but unlike the roots they did not grow well in aerated water where the oxygen was 20 p.p.m. This suggests that tissues within a stem may not all be equally affected by a given oxygen supply. Hahn, Hartley, and Rhodes (9) noted that hypertrophied lenticels of conifer roots were produced in the presence of excessive moisture but suspected that they might be related to a deficiency of oxygen. Since cuttings immersed in water high in oxygen do not produce hypertrophied lenticels, water alone is not the factor which induces this growth. Water with a low oxygen content (1 to 2 p.p.m.), however, stimulates the cells which produce hypertrophies. Two sets of tissues within a single stem have, therefore, two different oxygen requirements for growth.

The cuttings from different species tested show different oxygen requirements as reported by various authors for growth of seedlings in soil. Tomato, salvia, and ivy have a high oxygen requirement while willow roots can grow when the oxygen is only 1 p.p.m. When enough oxygen is available to permit of normal growth, the supplying of more oxygen does not cause increased growth.

#### SUMMARY

1. *Salvia* cuttings formed roots at the base of the stems that were placed in two inches of water. Similar cuttings in five-inch depths of water had a tendency to root along the stems toward the surface of the water. *Salvia* cuttings in water seven inches deep were either slow to respond or did not root at all.

2. *Salvia* cuttings aerated with oxygen produced basal roots in shallow and deep water alike.

3. A thin film of paraffin oil on the surface of the water prevents rooting by interfering with absorption of oxygen from the air.

4. *Salvia* cuttings in water covered with a thin film of paraffin oil formed roots readily when the water was aerated with oxygen.

5. *Salix pendula* cuttings formed roots at the base of the stems when in shallow water, but when placed in deep water (eight inches or more) roots

formed on the stems near the surface of the water. Cuttings in aerated water formed roots at the base of the stems and practically none at the surface of the water. A deficiency of oxygen at the bottom of a deep water column disturbed the natural polarity of willow cuttings.

6. Analyses indicate that willow cuttings will form roots in water if the oxygen is 1 p.p.m. or more, this being the amount frequently found at the surface of a deep water column where roots were growing. Practically no oxygen could be found at the bottom of a 15 inch column of water in which ten cuttings were growing. Analyses of aerated water showed 20 to 30 p.p.m. at all depths.

7. Hypertrophied lenticels were produced more abundantly in non-aerated water than in aerated water. As with roots, excrescences varied from the surface of the water, becoming less and less toward the bottom of the column where the oxygen was practically depleted.

8. Running water was in effect equal to aeration due to the fact that the normal oxygen content of tap water was higher than the optimum amount required by the different species used.

9. English ivy required a higher oxygen supply for root growth than willow. Where the oxygen was 4 p.p.m. the root growth was less than where the supply was 20 p.p.m.

10. The oxygen supply in non-aerated water surrounding tomato cuttings varied with the depth of the water column and also with exposure to light. Cuttings in cylinders wrapped with black paper soon depleted the water of oxygen while those in cylinders exposed to light aerated the water through photosynthesis.

11. Tomato cuttings in deep water disintegrate readily if the cylinders containing the water are wrapped with black paper or the whole lot is kept in a dark room.

12. Callus forms on Dorothy Perkins rose cuttings in shallow water but not in deep water.

13. Oxidizing agents such as hydrogen peroxid and potassium permanganate increased the amount of rooting but they were not so effective as aeration.

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## INDEX TO VOLUME II

---

- Abies arizonica*: germination of seeds, 317, 327, 333, 337
- Acetaldehyde: effect of chemicals on formation in potatoes, 437
- Acidity: See Hydrogen-ion concentration
- Adventitious roots: See Roots
- Aeration: effect on callusing of apple cuttings and grafts, 374, 385
- Aeration: Oxygen requirements for root growth of cuttings in water, 616
- After-ripening: Primary dormancy, after-ripening, and development of secondary dormancy in embryos of *Ambrosia trifida*, 285
- After-ripening: birch seeds, 47; *Xanthium* seeds, 304
- Age of plants: effect on chemical composition, 467
- Alternating temperature effects: on callusing of apple cuttings and grafts, 351; on germination of *Ambrosia trifida*, 292, birch, 47, parsnip, 115
- Ambrosia artemisiifolia*: effect of light duration, 502
- Ambrosia trifida*: Primary dormancy, after-ripening, and development of secondary dormancy in embryos of *Ambrosia trifida*, 285
- Amphicarpa monoica*: effect of light, 170
- Amylase of potatoes: effect of acidity on, 435, chemicals on, 435
- Antirrhinum*: effect of aspirin on flowers, 197; effect of carbon dioxide, light, 502
- Antirrhinum majus*: effect of carbon dioxide on flowers, 544
- Apple: See *Pyrus*
- Arbor vitae: See *Thuja*
- Arthur, John M., Guthrie, John D., and Newell, John M. Some effects of artificial climates on the growth and chemical composition of plants, 445
- Arthur, John M., and Newell, John M. The killing of plant tissue and the inactivation of tobacco mosaic virus by ultra-violet radiation, 143
- Asparagus plumosus*: effect of carbon dioxide storage, 544
- Aspidium spinulosum*: effect of carbon dioxide storage, 544
- Aspirin: effect on flowers, 197
- Aster*: effect of chemicals on flowers, 197
- Aster*, China: See *Callistephus chinensis*
- Aster* yellows: examination of *Cicadula sexnotata* for virus, 343
- Avena*: effect of carbon dioxide, light, 497
- Avens: See *Geum canadense*
- Barley: See *Hordeum*
- Barton, Lela V. Hastening the germination of some coniferous seeds, 315
- Bean: See *Phaseolus*
- Bean mosaic: comparison with tobacco mosaic on bean varieties, 556
- Betula lenta*, *lutea*, *papyrifera*, *populifolia*: Germination and vitality of birch seeds, 47
- Birch: See *Betula*
- Black grain thrips: See *Heliothrips femoralis*
- Blindness: *Gladiolus*, 532
- Botrytis*: effect of pentathionic acid, sulfuric acid, and hydrogen sulfide on germination of spores, 389
- Brassica oleracea*: effect of carbon dioxide, light, 485
- Brassica rapa*: fumigation with naphthalene, 518
- Bryophyllum*: fumigation with naphthalene, 518
- Buckwheat: See *Fagopyrum esculentum*
- Cabbage: See *Brassica oleracea*
- Calendula officinalis*: fumigation with naphthalene, 518
- Callistephus chinensis*: fumigation with naphthalene, 518
- Callus: effect of oxygen on formation on cuttings, 616; on apple cuttings and grafts, 351



- Capsicum annuum*: fumigation with naphthalene, 518
- Carbohydrates: effect of artificial climates on plant composition, 445; effect of seed composition on growth of seedlings, 1, 251, 274; in holly cuttings, 212
- Carbohydrate-nitrogen ratio: effect of artificial climates on plant composition, 445; effect of seed composition on growth of seedlings, 1, 251
- Carbon dioxid effects: on callusing of apple cuttings and grafts, 378, 385; on chloroplast pigments, 228; on germination of birch seeds, 69; on growth of seedlings, 1, 251, 274; on plant growth, 445; on prolonging life of cut flowers, 535
- Carlson, Margery C. Origin of adventitious roots in *Coleus* cuttings, 39
- Carnation: See *Dianthus carophyllus*
- Carotin: effect of environment on, 220; preparation, 226
- Carrot: See *Daucus carota*
- Catalase: in *Ambrosia trifida* embryos, 291, 298; of potatoes, effect of chemicals on, 420, 427; in *Xanthium* seeds, 310
- Cedar, incense: See *Libocedrus decurrens*
- Centaurea imperialis*: fumigation with naphthalene, 518
- Chemical changes induced in potato tubers by treatments that break the rest period, F. E. Denny, 131
- Chemicals: Shortening rest period of *Gladiolus* by treatment with chemicals, 523
- Chemicals, effects: on enzym activities in potatoes, 417; on flowers, 197
- Chlorophenol mercury: effect on birch seeds, 52
- Chlorophyll: determination of chlorophyll a and b, 222; effect of environment on, 220; effect of light on concentration, 184, 189
- Chloroplast pigments: determinations, 224; effect of environment on, 220; effect of tomato mosaic on, 244
- Chrysanthemum*: effect of aspirin on flowers, 197; effect of oxygen supply on rooting of cuttings, 616
- Cicadula sexnotata*: examination for aster-yellows virus, 343
- Climates: effect on plants, 445
- Clover, red: See *Trifolium pratense*
- Clover, white: See *Trifolium repens*
- Cocklebur: See *Xanthium*
- Coleus*: effect of carbon dioxid, light, 504
- Coleus blumei*: Origin of adventitious roots in *Coleus* cuttings, 39
- Coleus blumei*: effect of oxygen supply on rooting of cuttings, 616
- Conifers: germination of seeds, 315
- Coreopsis* flowers: effect of chemicals, 197, temperature, 200
- Corms, *Gladiolus*: effect of chemicals on formation, 527
- Corn: See *Zea*
- Cosmos* flowers: effect of aspirin, 197, carbon dioxid, 544, humidity, 200, temperature, 200
- Cotyledon growth: effect of light, seed composition, 270
- Cow pea: See *Vigna sinensis*
- Cucumber: See *Cucumis sativus*
- Cucumis melo* growth: effect of light, 1, 255, seed composition, 255
- Cucumis sativus*: effect of carbon dioxid, light, 491; fumigation with naphthalene, 518
- Cucurbita maxima*: effect of light on growth in relation to available nitrogen and carbon, 1
- Cupressus macrocarpa*: germination of seeds, 317, 327, 333, 337
- Cut flowers: See Flowers
- Cuttings: Oxygen requirements for root growth in water, 616
- Cuttings, apple: effect of environment on callusing, 351
- Cuttings, *Coleus*: origin of adventitious roots, 39
- Cuttings, holly: 205
- Cyclamen indicum*: fumigation with naphthalene, 518
- Cyclamen* mite: See *Tarsonemus pallidus*
- Cypress: See *Cupressus*
- Cypress, bald: See *Taxodium distichum*
- Cysteine: effect of potato juice on oxidation, 558
- Dahlia* flowers: effect of aspirin, 197, carbon dioxid, 544
- Datura stramonium*: fumigation with naphthalene, 518

- Daucus carota*: fumigation with naphthalene, 518
- Davis, W. E. The development of dormancy in seeds of cocklebur (*Xanthium*), 304; Primary dormancy, after-ripening, and the development of secondary dormancy in embryos of *Ambrosia trifida*, 285
- Day length: See Photoperiodism
- Delphinium*: effect of chemicals on flowers, 197
- Denny, F. E. Chemical changes induced in potato tubers by treatments that break the rest period, 131; Rôle of mother tuber in growth of potato plant, 77; Shortening the rest period of *Gladiolus* by treatment with chemicals, 523; Sucrose and starch changes in potatoes treated with chemicals that break the rest period, 580; The twin-leaf method of studying changes in leaves, 592
- Denny, F. E., Miller, Lawrence P., and Guthrie, John D. Enzym activities of juices from potatoes treated with chemicals that break the rest period, 417
- Development of dormancy in seeds of cocklebur (*Xanthium*), W. E. Davis, 304
- Dialysis: effect on potato juice, 424
- Dianthus carophyllus* flowers: effect of carbon dioxid, 544, humidity, 200, temperature, 200
- Dobroscky, Irene D. Is the aster-yellows virus detectable in its insect vector? 343
- Dormancy: Chemical changes induced in potato tubers by treatments that break rest period, 131; Development of dormancy in seeds of cocklebur (*Xanthium*), 304; Enzym activities of juices from potatoes treated with chemicals that break rest period, 417; Primary dormancy, after-ripening, and development of secondary dormancy in embryos of *Ambrosia trifida*, 285; Shortening rest period of *Gladiolus* by treatment with chemicals, 523; Sucrose and starch changes in potatoes treated with chemicals that break rest period, 580
- Dormancy: causes, 300; in birch seeds, 47 "Dry ice": See Carbon dioxid
- Duplicate determinations: calculation of probable error from, 226
- Ecological significance of light intensity, 191
- Effect of chemicals, temperature, and humidity on the lasting qualities of cut flowers, A. E. Hitchcock and P. W. Zimmerman, 196
- Effect of environmental conditions on the chloroplast pigments, John D. Guthrie, 220
- Effect of variations in the amounts of available carbon and nitrogen on the growth of wheat seedlings, Mary E. Reid, 274
- Egg plant: See *Solanum melongena*
- Embryo: See Seeds
- Enzym activities of juices from potatoes treated with chemicals that break the rest period, F. E. Denny, L. P. Miller, and John D. Guthrie, 417
- Enzymes: See Amylase; Invertase; Peroxidase; Reducing enzymes
- Ethylene: effect on rest period of *Gladiolus*, 523
- Ethylene chlorhydrin effects: on enzym activities in potatoes, 417; on potato tubers, 131; on rest period of *Gladiolus*, 523; on starch content of potatoes, 581; on sucrose content of potatoes, 581
- Ether: effect on rest period of *Gladiolus*, 523
- Fagopyrum esculentum*: effect of light, 170, 460; fumigation with naphthalene, 518
- Ferns, asparagus: See *Asparagus plumosus*
- Ferns, broad frond: See *Aspidium spinulosum*
- Fir: See *Abies*
- Flowering: effect of carbohydrate-nitrogen ratio, 466, chemical composition of plants, 475, light duration, 466, 478, light intensity, 185
- Flowers, *Gladiolus*: effect of chemicals on production, 531
- Flowers, lasting qualities: effect of carbon dioxid, 535, chemicals, humidity, temperature, 196
- Forsythia intermedia*: effect of oxygen supply on rooting of cuttings, 616

- Frasch foundation: See Herman Frasch foundation for research in agricultural chemistry
- Fruiting: effect of light intensity, 185, of ultra-violet on tomatoes, 157
- Fuchsia speciosa*: fumigation with naphthalene, 518
- Fumigation: with naphthalene, 512
- Fungicidal action of sulfur: I. The alleged rôle of pentathionic acid, Frank Wilcoxon and S. E. A. McCallan, 389
- Galinsoga parviflora*: effect of light, 170
- Geranium*: effect of carbon dioxide, light, 504; See also *Pelargonium* sp.
- Germination and keeping quality of parsnip seeds under various conditions, Hilda C. Joseph, 115
- Germination and vitality of birch seeds, Hilda C. Joseph, 47
- Germination: Chemical changes induced in potato tubers by treatments that break rest period, 131; Hastening germination of some coniferous seeds, 315; Shortening rest period of *Gladiolus* by treatment with chemicals, 523
- Germination: *Ambrosia trifida* embryos, 285; spores treated with hydrogen sulfide, pentathionic acid, sulfuric acid, 389; *Xanthium* seeds, 304
- Geum canadense*: effect of light, 170
- Gladiolus*: shortening rest period by chemical treatments, 523
- Gladiolus* flowers: effect of carbon dioxide, 544, chemicals, 197, humidity, 200, temperature, 200
- Glycine soja*: chloroplast pigments, 228; effect of carbon dioxide, 491, light, 1, 255, 491, seed composition, 255; fumigation with naphthalene, 518
- Grafts, apple: effect of environment on callusing, 351
- Greenness of leaves: relation to conditions of growth, 281
- Growth of seedlings in light and in darkness in relation to available nitrogen and carbon, Mary E. Reid, 1
- Growth: Effect of variations in amounts of available carbon and nitrogen on growth of wheat seedlings, 274; Influence of light intensity and light quality upon growth of plants, 159; Relation of composition of seed and effects of light on growth of seedlings, 251; Rôle of mother tuber in growth of potato plant, 77; Some effects of artificial climates on growth and chemical composition of plants, 445; Vegetative propagation of holly, 213
- Growth: effect of chemical treatment on potatoes, 131, ultra-violet on tomatoes, 157
- Guthrie, John D. Effect of environmental conditions on the chloroplast pigments, 220; An iodimetric method for determining oxidase activity, 558; See also Arthur, John M., Guthrie, John D., and Newell, John M.; Denny, F. E., Guthrie, John D., and Miller, Lawrence P.
- Hartzell, Albert, and Wilcoxon, Frank. Naphthalene fumigation at controlled concentrations, 512
- Hastening the germination of some coniferous seeds, Lela V. Barton, 315
- Hedera helix*: effect of oxygen supply on rooting of cuttings, 616
- Helianthus cucumerifolius*: chloroplast pigments, 228; effect of light on growth, 1, 170, 255; effect of seed composition, 255
- Heliothrips femoralis*: fumigation with naphthalene, 519
- Herman Frasch foundation for research in agricultural chemistry. Paper: no. 2, 389; no. 3, 417; no. 4, 512; no. 5, 523; no. 6, 558; no. 10, 580
- Hitchcock, A. E., and Zimmerman, P. W. Effect of chemicals, temperature, and humidity on the lasting qualities of cut flowers, 196; See also Zimmerman, P. W., and Hitchcock, A. E.
- Hog peanut: See *Amphicarpa monoica*
- Holcus sudanensis*: fumigation with naphthalene, 518
- Holly: See *Ilex*
- Holmes, Francis O. Local and systemic increase of tobacco mosaic virus, 563
- Hordeum*: effect of carbon dioxide, light, 497
- Hordeum sativum*: effect of light on growth in relation to available nitrogen and carbon, 1
- Humidity: Inexpensive and quickly made

- instrument for testing relative humidity, 72
- Humidity effects: on lasting qualities of cut flowers, 200; See also Water effects
- Hydrogen-ion concentration: of *Ambrosia trifida* embryos, 291; effect of chemicals on potatoes, 417; effect on amylase in potatoes, 435, on callusing of apple cuttings and grafts, 351, on germination of birch seeds, 50, on holly propagation, 205, on invertase in potatoes, 435, on potato oxidase, 560
- Hydrogen peroxid: effect on root growth of cuttings in water, 616
- Hydrogen sulfide: rôle in fungicidal action of sulfur, 389; toxicity, 398
- Hypocotyl growth: effect of light, seed composition, 270
- Ilex*: vegetative propagation, 205
- Indophenol reduction, of potatoes: effect of chemicals on, 431
- Inexpensive and quickly made instrument for testing relative humidity, William B. Shippy, 72
- Influence of environment on the callusing of apple cuttings and grafts, William B. Shippy, 351
- Influence of light intensity and light quality upon the growth of plants, Hardy L. Shirley, 159
- Invertase, of potatoes: effect of chemicals on, 435
- Iodimetric method for determining oxidase activity, John D. Guthrie, 558
- Iodine reduction, by potato juice: effect of chemicals on, 432
- Is the aster-yellow virus detectable in its insect vector? Irene D. Dobrowsky, 343
- Ivy, English: See *Hedera helix*
- Joseph, Hilda C. Germination and keeping quality of parsnip seeds under various conditions, 115; Germination and vitality of birch seeds, 47
- Killing of plant tissue and the inactivation of tobacco mosaic virus by ultra-violet radiation, John M. Arthur and John M. Newell, 143
- Lactuca sativa*: effect of light duration on carbohydrate-nitrogen ratio, 460
- Lathyrus odoratus*: effect of carbon dioxide, light, 502; effect of carbon dioxide on flowers, 544
- Leafhopper: See *Cicadula scirnotata*
- Leaves: Twin-leaf method of studying changes in leaves, 592
- Leaves: effect of available carbon and nitrogen on growth of seedlings, 1, 251, 274; effect of light, 184, 187, 188, 270, of seed composition, 270; effect on holly propagation, 210, 215
- Length of day: See Photoperiodism
- Lenticels: effect of oxygen on development, 625
- Lettuce: See *Lactuca sativa*
- Libocedrus decurrens*: germination of seeds, 317, 327, 333, 337
- Light: Growth of seedlings in light and in darkness in relation to available nitrogen and carbon, 1; Influence of light intensity and light quality upon growth of plants, 159; Relation of composition of seed and effects of light to growth of seedlings, 251
- Light: long day plants, 462; short day plants, 462
- Light duration: effect on plants, 445
- Light effects: on chloroplast pigments, 220; on germination of birch seeds, 52; on holly propagation, 210; on oxygen content of water in which green cuttings were rooting, 629
- Light injury: *Colerus*, 504; *Geranium*, 504; tomato, 480
- Light intensity: ecological significance, 191; effect on plants, 159, 445
- Light quality: effect on plant growth, 159
- Light transmission: through glass filters, 167
- Light, artificial: use to supplement daylight, 446
- Ligustrum ovalifolium*: effect of oxygen supply on rooting of cuttings, 616
- Lilium rubrum*: effect of aspirin on flowers, 197
- Local and systemic increase of tobacco mosaic virus, Francis O. Holmes, 563
- Local lesions on bean leaves inoculated with tobacco mosaic virus, W. C. Price, 549

- Lycopersicum esculentum*: chloroplast pigments, 228; effect of carbon dioxide, 476, light, 1, 170, 455, 476, oxygen supply on rooting of cuttings, 616, ultra-violet, 143; fumigation with naphthalene, 518; movement of tobacco mosaic virus in stem, 574
- Macrosporium sarcinaeforme*: effect of pentathionic acid, sulfuric acid, and hydrogen sulfide on germination of spores, 389
- McCallan, S. E. A. See Wilcoxon, Frank, and McCallan, S. E. A.
- Membranes: effect on *Ambrosia trifida*, 285
- Methylene blue reduction, by potato juice: effect of chemicals on, 430
- Miller, Lawrence P. See Denny, F. E., Miller, Lawrence P., and Guthrie, John D.
- Moisture: See Water
- Mosaic diseases: See Bean mosaic; Tobacco mosaic; Tomato mosaic
- Multiple sprouts: See Sprouts
- Muskmelon: See *Cucumis melo*
- Naphthalene fumigation at controlled concentrations, Albert Hartzell and Frank Wilcoxon, 512
- Nasturtium: See *Tropaeolum*
- Newell, John M. See Arthur, John M., Guthrie, John D., and Newell, John M.; also Arthur, John M., and Newell, John M.
- Nicotiana*: Local and systemic increase of tobacco mosaic virus, 563
- Nicotiana*: comparison of *tabacum* and *glutinosa* as to susceptibility to tobacco mosaic, 565; transfer of tobacco mosaic virus from bean to tobacco, 552
- Nicotiana tabacum*: effect of carbon dioxide, 504, light, 170, 504; fumigation with naphthalene, 518; inactivation of tobacco mosaic virus by ultra-violet, 155
- Nitrates, effects: on chemical composition of plants, 470, 497; on chloroplast pigments, 241; on growth of seedlings, 1, 251, 274
- Nitrogen: effect on callusing of apple cuttings and grafts, 374, 385
- Nitrogen compounds: effect of artificial climates on plant composition, 445; effect of seed composition on growth of seedlings, 1, 251, 274; in potato tubers, 131; utilization by potato sprouts, 97
- Oats: See *Avena*
- Onion thrips: See *Thrips tabaci*
- Origin of adventitious roots in *Coleus* cuttings, Margery C. Carlson, 39
- Oxalis*: fumigation with naphthalene, 518
- Oxidase activity: effect of enzyme concentration, 560; iodimetric method for determining, 558
- Oxidizing agents: See Hydrogen peroxid; Potassium permanganate
- Oxygen requirements for root growth of cuttings in water, P. W. Zimmerman, 616
- Oxygen: micro-determination in water, 617
- Oxygen, effects: on *Ambrosia trifida* embryos, 290, 298; on callusing of apple cuttings and grafts, 351; on dormancy of *Xanthium* seeds, 311; on germination of birch seeds, 50; on parsnip seeds, 124; on roses, 535
- Parsnip: See *Pastinaca sativa*
- Pastinaca sativa*: Germination and keeping quality of parsnip seeds under various conditions, 115
- Pea: See *Pisum*
- Peach: See *Prunus persica*
- Peat moss effects: on callusing of apple cuttings and grafts, 351; on germination of birch seeds, 50; on holly propagation, 205
- Pelargonium*: fumigation with naphthalene, 518
- Pentathionates: preparation, 395; toxicity, 407
- Pentathionic acid: rôle in fungicidal action of sulfur, 389; toxicity, 394
- Pepper, red: See *Capsicum*
- Peroxidase, of potatoes: effect of chemicals on, 426
- Petunia*: effect of carbon dioxide, light, 502
- Phaseolus*: effect of light, 1, soil moisture, 170
- Phaseolus vulgaris*: fumigation with naphthalene, 518; inoculation with tobacco mosaic virus, 549; use in determination

- of tobacco mosaic virus concentration, 549; varieties susceptible to tobacco mosaic, 550
- Philadelphus*: effect of oxygen supply on rooting of cuttings, 616
- Phlox*: effect of chemicals on flowers, 197
- Phosphates: effect on chloroplast pigments, 241
- Phosphotungstic reagent reduction, in potatoes: effect of chemicals on, 433
- Photoperiodism: effect on plant growth, 445
- Photosynthesis: Twin-leaf method of studying changes in leaves, 592
- Physalis francheti*: fumigation with naphthalene, 518
- Picea*, germination of seeds: *canadensis*, 317, 328, 333, 337; *excelsa*, 317, 328, 333, 337; *omorika*, 317, 328, 333, 337; *pungens*, 317, 328, 333, 337; *sitchensis*, 317, 328, 333, 338
- Pigment: effect of chemicals on potato juice, 431
- Pigment, brown, soluble in dilute acetone: determination, 224
- Pigment, chloroplast: effect of environment, 220; tomato mosaic, 244
- Pine: See *Pinus*
- Pine, loblolly: See *Pinus taeda*
- Pinus*, germination of seeds: *austriaca*, 316, 317, 333, 338; *banksiana*, 317, 318, 333, 338; *cembra*, 317, 318; *contorta*, 317, 318, 319, 334, 338; *coulteri*, 317, 319, 334, 339; *densiflora*, 317, 319, 334, 339; *excelsa*, 317, 320, 334, 339; *flexilis*, 317, 321, 334, 339; *insignis*, 317, 321, 335, 340; *koraiensis*, 317, 321, 335, 340; *lambertiana*, 317, 322, 335, 340; *laricio*, 317, 323, 335, 340; *monticola*, 317, 323, 335, 341; *ponderosa*, 317, 324, 335, 341; *resinosa*, 317, 324, 336, 341; *rigida*, 317, 325, 336, 341; *strobus*, 317, 325, 336, 342; *thunbergii*, 317, 327, 336, 342
- Pinus taeda*: effect of light, 170
- Pisum sativum*: effect of light on growth in relation to available nitrogen and carbon, 1
- Plant composition: effect of artificial climates, 445
- Polarity: effect on callusing of apple cuttings and grafts, 357, 378, 385
- Portulaca oleracea*: effect of oxygen supply on rooting of cuttings, 616
- Potassium: effect on chloroplast pigments, 241
- Potassium permanganate: effect on root growth of cuttings in water, 616
- Potato: See *Solanum tuberosum*
- Price, W. C. Local lesions on bean leaves inoculated with tobacco mosaic virus, 549
- Primary dormancy, after-ripening, and the development of secondary dormancy in embryos of *Ambrosia trifida*, W. E. Davis, 285
- Privet: See *Ligustrum ovalifolium*
- Probable error: calculation from duplicate determinations, 226
- Propagation: of holly, 205
- Protein: effect of seed composition on growth of seedlings, 1, 251, 274
- Prunus persica*: fumigation with naphthalene, 518
- Prunus tomentosa*: effect of oxygen supply on rooting of cuttings, 616
- Pyrus*: Influence of environment on callusing of apple cuttings and grafts, 351
- Radiation, solar: required for plant growth, 179; See also Light; Ultraviolet
- Radish: See *Raphanus*
- Ragweed: See *Ambrosia*
- Raphanus*: effect of light duration on carbohydrate-nitrogen ratio, 460
- Raphanus sativus*: fumigation with naphthalene, 518
- Red spider mite: See *Tetranychus telarius*
- Reducing enzymes, of potatoes: effect of chemicals on, 435
- Redwood, California: See *Sequoia sempervirens*
- Reid, Mary E. Effect of variations in the amounts of available carbon and nitrogen on the growth of wheat seedlings, 274; Growth of seedlings in light and in darkness in relation to available nitrogen and carbon, 1; Relation of composition of seed and the effects of light to growth of seedlings, 251
- Relation of composition of seed and the effects of light to growth of seedlings, Mary E. Reid, 251

- Relative humidity: Inexpensive and quickly made instrument for testing relative humidity, 72; See also Water
- Respiration: *Ambrosia trifida* embryos, 296; *Xanthium* seeds, 310
- Rest period: See Dormancy
- Ring-spot of tobacco: comparison with tobacco mosaic in bean varieties, 557
- Ripeness effects: on germination of birch seeds, 47; on parsnip seeds, 119
- Rôle of mother tuber in growth of potato plant, F. E. Denny, 77
- Rooting: effect of oxygen on cuttings in water, 616
- Roots: Growth of seedlings in light and in darkness in relation to available nitrogen and carbon, 1; Origin of adventitious roots in *Coleus* cuttings, 39; Vegetative propagation of holly, 205
- Roots: effect of available carbon and nitrogen, 274, light, 182, 251, seed composition, 251
- Rosa*: effect of carbon dioxide, light, 502; fumigation with naphthalene, 518
- Rosa* Dorothy Perkins: effect of oxygen supply on rooting of cuttings, 616
- Rosa* flowers: effect of carbon dioxide, 535, humidity, 200, temperature, 200
- Rose: See *Rosa*
- Rye: See *Secale*
- Salix pendula*: effect of oxygen supply on rooting of cuttings, 616
- Salvia*: Twin-leaf method of studying changes in leaves, 592
- Salvia*: effect of day length on carbohydrate-nitrogen ratio, 460
- Salvia splendens*: effect of oxygen supply on rooting of cuttings, 616
- Sampling of plants: errors, 455
- Sand, effects: on callusing of apple cuttings and grafts, 351; on holly propagation, 205
- Sciadopitys verticillata*: germination of seeds, 317, 328, 336, 342
- Sclerotinia americana*: effect of pentathionic acid, sulfuric acid, and hydrogen sulfide on germination of spores, 389
- Seasonal effects: on germination of birch seeds, 47; on holly propagation, 207
- Secale cereale*: effect of light on growth in relation to available nitrogen and carbon, 1
- Sedum*: fumigation with naphthalene, 518
- Seed coats: effect on dormancy in *Xanthium*, 304; See also Membranes
- Seedling growth: effect of available carbon and nitrogen, 1, 251, 274, carbon dioxide, 1, 251, 274, light, 1, 251, seed composition, 1, 251, 274
- Seeds: Development of dormancy in seeds of cocklebur (*Xanthium*), 304; Effect of variations in amounts of available carbon and nitrogen on growth of wheat seedlings, 274; Germination and keeping quality of parsnip seeds under various conditions, 115; Hastening germination of some coniferous seeds, 315; Primary dormancy, after-ripening, and development of secondary dormancy in embryos of *Ambrosia trifida*, 285; Relation of composition of seeds and effects of light to growth of seedlings, 251
- Sequoia sempervirens*: effect of light, 170; germination of seeds, 317, 329
- Shade: See Light intensity
- Shippy, William B. An inexpensive and quickly made instrument for testing relative humidity, 72; Influence of environment on the callusing of apple cuttings and grafts, 351
- Shirley, Hardy L. The influence of light intensity and light quality upon the growth of plants, 159
- Shoots: Growth of seedlings in light and in darkness in relation to available nitrogen and carbon, 1
- Shoots: effect of available carbon and nitrogen, 1, 251, 274, light, 1, 182, 251, seed composition, 1, 251, 274
- Shortening the rest period of *Gladiolus* by treatment with chemicals, F. E. Denny, 523
- Slag: effect on holly propagation, 205
- Snapdragon: See *Antirrhinum*
- Sodium thiocyanate effects: on enzyme activities in potatoes, 417; on potato tubers, 131; on starch content of potatoes, 581; on sucrose content of potatoes, 581
- Solanum melongena*: effect of carbon dioxide, light, 504; fumigation with naphthalene, 518
- Solanum tuberosum*: Chemical changes in

- duced in potato tubers by treatments that break rest period, 131; Enzym activities of juices from potatoes treated with chemicals that break rest period, 417; Rôle of mother tuber in growth of potato plant, 77; Sucrose and starch changes in potatoes treated with chemicals that break rest period, 580
- Solanum tuberosum*: chemical analysis of mother tuber, 94; effect of amputation of mother tuber, 77, light duration, 493, temperature, 493; fumigation with naphthalene, 518; iodimetric method for determining oxidase activity in potato juice, 558; relation of sprouting to enzym activity, 439
- Solanum tuberosum*, eye tissue in tuber: chemical changes in, 131; in relation to enzym activity, 437, 442
- Some effects of artificial climates on the growth and chemical composition of plants, John M. Arthur, John D. Guthrie, and John M. Newell, 445
- Soybean: See *Glycine soja*
- Spore germination: effect of hydrogen sulfide, pentathionic acid, sulfuric acid, 389; laboratory method for toxicity tests, 390
- Sprouts: *Gladiolus* treated with chemicals, 531
- Spruce: See *Picea*
- Squash, Hubbard: See *Cucurbita maxima*
- Starch: Sucrose and starch changes in potatoes treated with chemicals that break rest period, 580
- Starch content: effect on callusing of apple cuttings and grafts, 381; in potato tubers, 131; utilization by potato sprout, 97
- Stems: effect of light, 184, 188
- Sterilization: effect on birch seeds, 52
- Storage effects: on *Ambrosia trifida* embryos, 288; on birch seeds, 47; on seeds, 115
- Stratification effects on germination: of birch seeds, 47; of coniferous seeds, 315
- Sucrose and starch changes in potatoes treated with chemicals that break the rest period, F. E. Denny, 580
- Sucrose, in potato tubers: effect of chemical treatments on, 131
- Sudan grass: See *Holcus sudanensis*
- Sugar: in potato tubers, 131; utilization by potato sprout, 97
- Sulfur: rôle of pentathionic acid, sulfuric acid, and hydrogen sulfide in fungicidal action, 389
- Sulfur toxicity: 392; as influenced by particle size, 410; of water extracts, 409
- Sulfuric acid: rôle in fungicidal action of sulfur, 389; toxicity, 398
- Sunflower: See *Helianthus cucumerifolius*
- Sunlight: effect of ultra-violet in sunlight on tomatoes, 143
- Sweet pea: See *Lathyrus odoratus*
- Tarsonemus pallidus*: fumigation with naphthalene, 519
- Taxodium distichum*: germination of seeds, 317, 330, 336, 342
- Temperature effects: on *Ambrosia trifida* embryos, 285; on callusing of apple cuttings and grafts, 351; on chloroplast pigments, 228; on dormancy in *Xanthium* seeds, 304; on germination of birch seeds, 47, coniferous seeds, 315; on holly propagation, 211; on lasting qualities of cut flowers, 200, 535; on parsnip seeds, 115; on plant growth, 445; on rest period of *Gladiolus*, 523
- Tetranychus telarius*: fumigation with naphthalene, 519
- Thiourea effects: on enzym activities in potatoes, 417; on starch content of potatoes, 581; on sucrose content of potatoes, 581
- Thornton, Norwood C. Use of carbon dioxide for prolonging the life of cut flowers, with special reference to roses, 535
- Thrips tabaci*: fumigation with naphthalene, 519
- Thuja gigantea, occidentalis, orientalis*: germination of seeds, 317, 331, 336, 342
- Tissues: Killing of plant tissue and inactivation of tobacco mosaic virus by ultra-violet radiation, 143
- Tobacco: See *Nicotiana tabacum*
- Tobacco mosaic: Local and systemic increase of tobacco mosaic virus, 563; local lesions on bean leaves inoculated with tobacco mosaic virus, 549
- Tobacco mosaic: inactivation by ultra-violet, 155
- Tomato: See *Lycopersicon esculentum*



- Tomato mosaic: effect on chloroplast pigments, 244
- Tradescantia fluminensis*: effect of light, 170
- Translocation: Twin-leaf method of studying changes in leaves, 592
- Trifolium pratense*: effect of carbon dioxide, light, temperature. 487; fumigation with naphthalene, 519
- Trifolium repens*: fumigation with naphthalene, 519
- Triticum aestivum*: effect of available carbon, 1, 274. carbon dioxide, 1, 497, light, 1, 497; fumigation with naphthalene, 519
- Tropaeolum*: effect of carbon dioxide, light, 502
- Tropaeolum majus*: fumigation with naphthalene, 519
- Tuber: Rôle of mother tuber in growth of potato plant, 77
- Turnip: See *Brassica rapa*
- Twin-leaf method of studying changes in leaves, F. E. Denny, 592
- Ultra-violet: effect on tobacco mosaic virus, 143. on tomatoes, 143; filters for transmission, 146
- Umbrella-pine: See *Sciadopitys verticillata*
- Uromyces carophyllinus*: effect of pentathionic acid, sulfuric acid, and hydrogen sulfide on germination of spores, 389
- Use of carbon dioxide for prolonging the life of cut flowers, with special reference to roses, Norwood C. Thornton, 535
- Upsulun: effect on germination of birch seeds, 52
- Varietal differences: in respect to callusing of apple cuttings and grafts, 357, 382, 385
- Vegetative propagation of holly, P. W. Zimmerman and A. E. Hitchcock, 205
- Vicia faba*: inoculation with tobacco mosaic virus, 551
- Vigna sinensis*: effect of light on growth, 1, 255; effect of seed composition on growth, 255; inoculation with tobacco mosaic virus, 551
- Virus diseases: See Aster yellows; Bean mosaic; Ring-spot of tobacco; Tobacco mosaic; Tomato mosaic
- Vitality: Germination and keeping quality of parsnip seeds under various conditions, 115; Germination and vitality of birch seeds, 47
- Wandering jew, green: See *Tradescantia fluminensis*
- Wandering jew, purple: See *Zebrina pendula*
- Water: in mother tuber of potato plant, 97
- Water effects: on *Ambrosia trifida* embryos, 289; on callusing of apple cuttings and grafts, 351; on cut roses, 545; on dormancy of *Xanthium* seeds, 304; on germination of birch seeds, 47; on parsnip seeds, 119
- Wheat: See *Triticum*
- Wilcoxon, Frank, and Hartzell, Albert: See Hartzell, Albert, and Wilcoxon, Frank
- Wilcoxon, Frank, and McCallan, S. E. A. Fungicidal action of sulphur: I. The alleged rôle of pentathionic acid, 389
- Willow: See *Salix pendula*
- Xanthium*: Development of dormancy in seeds of cocklebur (*Xanthium*), 304
- Xanthophyll: effect of environment, 220
- Yellows disease: See Aster yellows
- Zea mays* growth: effect of light, 1, 255, of seed composition, 255
- Zebrina pendula*: effect of light, 170
- Zimmerman, P. W. Oxygen requirements for root growth of cuttings in water, 616
- Zimmerman, P. W., and Hitchcock, A. E. Vegetative propagation of holly, 205; See also Hitchcock, A. E., and Zimmerman, P. W.





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